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THE RAMI COMMUNICANTES IN THE RHESUS MONKEY

By D. SHEEHAN and J. PICK
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In a recent study of the small myelinated fibres in ventral spinal roots (Sheehan, 1941) the limits of the thoraco-lumbar outflow in the monkey were defined as T1/T2–L3/L4, rarely L5, and in man as T1–L2. The observations fitted into the general pattern of the sympathetic system, described first in the dog and cat by Gaskell (1886) and Langley (1894). In the rami communicantes, however, the small medullated fibres are distributed more widely. Not only do they take part in the constitution of the white rami of the thoraco-lumbar region, but they are also found in almost all the grey rami, particularly in those of the lower cervical and lower lumbar nerves. Langley (1896) was well aware of their existence within grey rami; the term 'grey', he remarked, is one of convenience rather than of anatomical accuracy. Kiss & Mihálik (1929) go so far as to deny any basic histological distinction between grey and white rami. Mixed rami are so common that, in their opinion, a pure grey or a pure white ramus is rarely seen. There are obviously widely possible differences in the interpretation of the microscopical appearances of sympathetic rami. Thus the number of myelinated fibres found in the rami of L3 and L4 led Müller (1909) to conclude that the thoraco-lumbar outflow extended in man as far down as the fourth lumbar segment. Further, a white ramus connected with the eighth cervical nerve has been mentioned as an occasional finding by Harman (1900) and Wrete (1934), and yet, in all examples that have been examined, its ventral spinal root is virtually devoid of the small myelinated fibres of the size constituting the thoraco-lumbar outflow.

What then are the criteria by which we distinguish a white ramus? Are the white rami distributed strictly within a thoraco-lumbar outflow as defined by a study of the ventral spinal roots? And what is the significance of the small myelinated fibres within rami, admittedly grey, and lying outside these limits? Langley (1896) and Nevin (1930) believed that the majority were postganglionic in nature, but Ranson & Billingsley (1918) and Kuntz & Farnsworth (1931) showed that some were afferent, at least with a cell station lying within dorsal spinal roots. That others, though a minority, might be preganglionic fibres passing to aberrant sympathetic nerve cells lying along grey rami or within spinal nerve roots, was first suggested by Langley (1896), and the hypothesis has recently been elaborated by Wrete (1935).

With these questions in mind we have undertaken a full histological investigation of the sympathetic rami in the monkey, in order to obtain a basis for comparison with the study of the ventral roots, already reported. There has apparently been no previous communication on the fibre constitution of the rami in the macaque. Gross anatomical studies have been undertaken by Broeck (1907–8) and by Botár (1932) in single examples of various species of monkey. In the rhesus monkey, dissections have been made of the cervical sympathetic chain by Riegele (1926), of the lumbo-sacral portion by Trumble (1933–4) and of the coccygeal region by Schumacher (1905), but by far the most complete study of the distribution of the rami communicantes in the macaque has been carried out by Zuckerman (1938), and the details of gross anatomy which

are reported incidentally in the present paper serve merely to supplement these earlier observations. A general account of the autonomic nervous system in the rhesus monkey by Kuntz (1933) is to be found in the monograph on the anatomy of this animal. No statistical data on the commoner variations have been reported.

The surgical importance of defining accurately the distribution of the white rami communicantes is of special significance to-day when a preganglionic section is being recommended as the operation of choice where sympathetic denervation of a part is desired. Should many of the small myelinated fibres in grey rami prove to be preganglionic, however, the anatomical basis for all operations on the sympathetic chain would be altered.

MATERIAL AND METHODS

The sympathetic chains together with the rami communicantes to spinal nerves have been dissected on both sides in twenty rhesus monkeys (*Macaca mulatta*). The communicating rami of the upper cervical nerves were only included in the last six preparations; otherwise the dissections were complete. The visceral and other rami were omitted as their dissection would have complicated and lengthened the study unduly. The animals had previously been used for experimental purposes and had been fixed immediately after death by intravascular injections of 10% formol-saline. The dissections were recorded by diagrams in each case.

In ten of the series, typical rami from various regions, particularly from the lower cervical and upper lumbar levels (boundaries of the thoraco-lumbar outflow), were removed and placed in 10% formol-saline for 24 hr. They were then washed and stained in bulk in 1% osmic acid, and sectioned in paraffin (5 μ thick). A certain amount of shrinkage undoubtedly occurred by this procedure, but as this applied to all fibres their relative sizes were not materially altered.

NOMENCLATURE

There are two possible methods of enumerating the ganglia in gross dissection. A ganglion might be numbered according either to the spinal nerve to which its grey ramus passed, or to that joined by its white ramus, as these terms are customarily defined. Zuckerman (1938) advocated the latter as the method of choice for embryological reasons but, in point of fact, a white ramus is not necessarily related in function to its corresponding sympathetic ganglion, for many of its fibres run through and terminate in other ganglia higher or lower in the chain. Indeed, the fibres entering the sympathetic trunk through one white ramus may be distributed to 5–10 successive ganglia. Furthermore, certain ganglia receive two and even three white rami from several spinal nerves. Enumeration of the ganglia according to the grey rami, on the other hand, seems to be more practical, since the grey ramus appears to connect a spinal nerve specifically with the ganglion of the same segment. This method was used by Langley (1896) and by Ranson & Billingsley (1918).

In the upper thoracic region the matter is simple, since the ganglia are each connected with only one spinal nerve. In the lower thoracic and upper lumbar regions, however, a ganglion is almost invariably connected with two or more spinal nerves. Botár (1932) has pointed out that the course of the white rami in the lumbar region is oblique, but in a gross dissection it is impossible without histological verification to distinguish a white from a grey ramus. In the present study, therefore, it was found necessary to lay down certain arbitrary rules, in order to bring about a uniformity in the descriptions

of the various dissections, and to permit accurate comparison when the microscopical studies were available.

Enumeration was started in each case with the ganglion connected with the third thoracic spinal nerve. This was constant and could be readily recognized. Naming this the third thoracic ganglion, enumeration followed downwards consecutively as far as the eleventh thoracic ganglion, which usually lay on the body of the twelfth thoracic vertebra. The ganglion immediately below this lay on the body of the first lumbar vertebra and was connected by rami with both the twelfth thoracic and first lumbar nerves, the former connexion subsequently proving to be the white ramus. Had the ganglia been enumerated according to the white rami, this would have been the twelfth thoracic, and the next lower ganglion would have been the first lumbar. The last lumbar and all the sacral ganglia would then have been numbered according to a spinal segment with which they had no connexion. The ganglion in question, located on the body of the first lumbar vertebra, and connected with both the twelfth thoracic and first lumbar nerves, was therefore called the first lumbar. Counting down in sequence now gave a regular enumeration of seven lumbar and three sacral ganglia. In only one monkey were thirteen ribs found, and Zuckerman's suggestion (1938) that the thirteenth thoracic nerve and its corresponding ganglion be called the first lumbar was followed; the total number of lumbar nerves then became eight as there was an extra vertebra in the thoraco-lumbar region.

At the upper end of the thoracic sympathetic chain the second thoracic nerve was either connected with the stellate ganglion or with a separate ganglion (the second thoracic). In the latter instance the stellate was formed by a fusion of the lower cervical and first thoracic ganglia only. In the other examples the second thoracic ganglion was incorporated within the stellate. Between the stellate and the superior cervical ganglion there was a small middle cervical ganglion placed invariably at the upper junction of the two limbs of the subclavian ansa.

This method of enumeration was satisfactory when a regular number of ganglia was encountered. Occasionally, however, it was obvious from the precise connexions of the rami and from the total number of ganglia present that there had been a fusion of two or more ganglia, or a splitting of one ganglion into two smaller masses. This usually occurred in the lumbo-sacral region. Such variations were taken into account in the method of enumeration, and described separately. Although considerable irregularity in the number and arrangement of the ganglia and rami existed, a definite pattern could be easily recognized, and the frequency of variation from this pattern defined.

FORM AND CONNEXIONS OF THE SYMPATHETIC CHAIN AND GANGLIA

The regular course of the sympathetic chains followed closely that outlined by Kuntz (1933) and Zuckerman (1938), and needs no further description.

Some form of transverse connexion between the sympathetic chains of each side was a constant feature in every dissection. Two types were generally recognized, one in the form of a complete fusion of the corresponding ganglia of each side, and the other as short nerve strands connecting the two chains. Both types were found present in the same dissection at different levels, the short intercommunicating branches more often in the lower lumbar and upper sacral regions, the fused ganglia more often at the termination of the two chains over the coccyx. In one instance the lumbar chains

were placed unusually close together on the ventral surface of the vertebral bodies, and fusion of the fifth lumbar ganglion of each side occurred in the midline. Transverse connexions never occurred above the level of the fourth lumbar vertebra. In the sacral region, a ganglion on one side occasionally gave a ramus to the corresponding spinal nerve of the opposite side, forming another, rather unusual, type of transverse connexion.

The sympathetic chain was rarely double, only eleven times in forty sides dissected. It occurred between the levels of the twelfth thoracic and first sacral vertebrae, and was therefore a peculiarity of the lumbar sympathetic chain. A doubled chain never extended for any greater distance than the interval between two adjacent ganglia, so that in an experimental extirpation of the lumbar chain it would not be easily overlooked.

In the more regular examples there were, according to the method of enumeration adopted here, three cervical, eleven thoracic (including those thoracic ganglia incorporated within the stellate), seven lumbar, three sacral and one or more coccygeal ganglia. The total number in any particular case might be altered in three ways: (*a*) by *fusion* of two adjacent ganglia, (*b*) by *absence* of a ganglion (revealed by the rami arising from the chain directly), and (*c*) by *duplication* of a ganglion. Because more than one of these conditions was frequently present, the simple recording of the total number of ganglia in any one instance has no special significance. Thus a count of twelve thoracic ganglia would not mean a special extra ganglion for the twelfth thoracic nerve, but frequently a duplication of the ninth or tenth thoracic ganglion. In the lumbar region the number of ganglia varied from six to eight, with duplication of the fourth lumbar occurring in ten instances.

Superior and middle cervical ganglia. The superior cervical ganglion was regular and placed in all instances opposite the bodies of the upper two cervical vertebrae. The upper pole could be clearly defined between the jugular and carotid foramina. Rami were traced to the upper three cervical nerves, usually only one to each nerve.* Several rami connected the superior cervical ganglion with the ninth, tenth, eleventh, and twelfth cranial nerves, and in one or two instances the separation of the ganglion from the nodose ganglion of the vagus was difficult.

The superior cervical ganglion was connected by a long thin nerve trunk, the cervical sympathetic cord, with the middle cervical ganglion, placed usually opposite the intervertebral disc between the seventh cervical and first thoracic vertebrae, and immediately above the first part of the subclavian artery. The left middle cervical ganglion was therefore generally lower than the right. The two limbs of the subclavian ansa joined the middle cervical ganglion with the stellate. Neither from the cervical sympathetic cord nor from the middle cervical ganglion were any rami communicantes traced to the cervical nerves. Communications with the vagus and its recurrent laryngeal branch were common. The constancy and regularity in position of the middle cervical ganglion in the monkey contrasted with the more variable conditions found in man (Axford, 1927–8; Siwe, 1931; Sheehan, 1933).

Stellate ganglion. In twenty-seven instances the stellate ganglion represented a fusion of the inferior cervical, first and second thoracic ganglia, and was situated opposite

* For purposes of description and before the histological preparations had been studied, the rami were referred to in the gross dissections as passing from a ganglion *to* a spinal nerve, realizing of course that conduction in its constituent fibres might be in the opposite direction.

the bodies of the first and second thoracic vertebrae. In twelve instances it was formed by the inferior cervical and first thoracic ganglia only, and did not extend caudally beyond the lower limits of the first thoracic vertebra. In these examples the next lower ganglion, connected with the second thoracic nerve, was clearly a separate second thoracic ganglion. In one exceptional arrangement two ganglia took the place of the

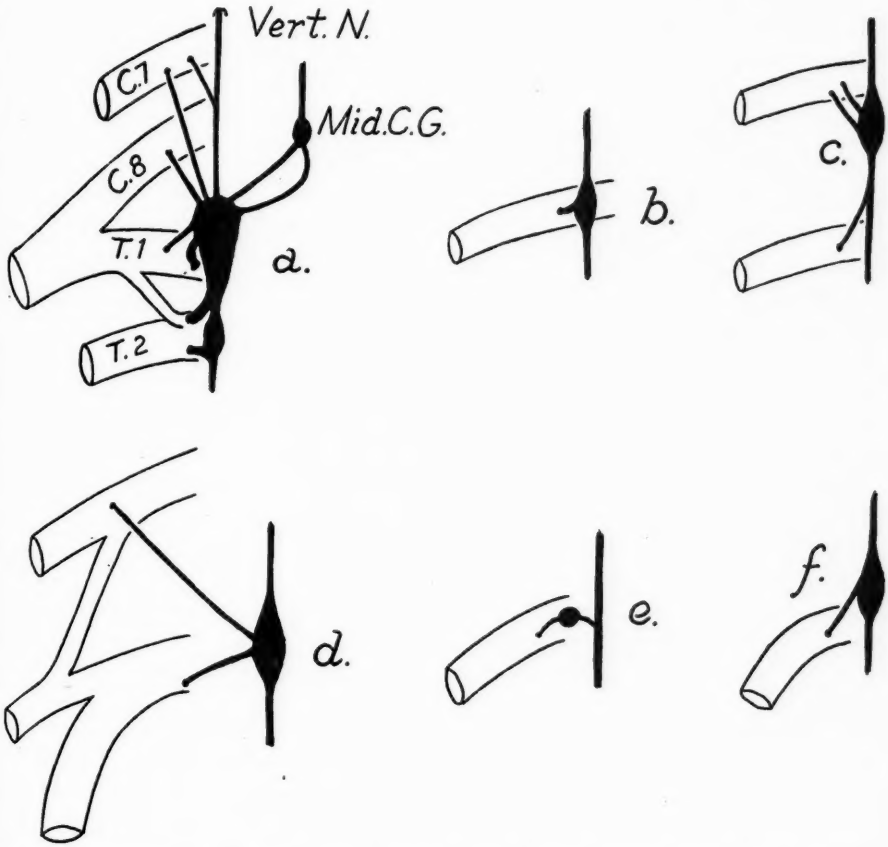

Fig. 1. Diagrammatic form of ganglia at different levels: (*a*) stellate; (*b*) upper thoracic (T3–T7); (*c*) lower thoracic (T8–T12); (*d*) upper lumbar (L1–L4); (*e*) intermediary ganglion on the course of a grey ramus (L2); (*f*) lower lumbar and sacral (L5–S3).

stellate; there was a small inferior cervical ganglion, and just below it a larger mass representing a fusion of the first and second thoracic ganglia. In the following analysis of the data concerning the rami of this region, the two ganglia in this exceptional case have been regarded as homologous with the stellate.

The communicating rami from the stellate ganglion were principally distributed to the nerves taking part in the brachial plexus (*a* in Fig. 1). They can be listed as follows:

(1) *Vertebral nerve*, present in all instances, and from which arose:

(*a*) Ramus to C8 (thirty-two out of forty instances).
(*b*) Ramus to C7 (thirty-six out of forty instances).
(*c*) Ramus to C6.
(*d*) Ramus to C5.
(*e*) Ramus to C4.

The vertebral nerve was traced through the transverse foramina of the cervical vertebrae in six dissections, and in every instance the rami to C6, C5 and C4 were present, although the last was often difficult to trace.

(2) *Rami to* C7 (in addition to those from the vertebral nerve):

None	20	instances
One ramus	17	,,
Two rami	3	,,
Total	40	,,

(3) *Rami to* C8 (in addition to those from the vertebral nerve):

None	15	instances
One ramus	20	,,
Two rami	3	,,
Three rami	1	,,
Four rami	1	,,
Total	40	,,

In those examples where no separate rami were given off to C7 or C8, the nerves invariably received a branch from the vertebral nerve (as described above).

(4) *Rami to* T1:

One ramus	10	instances
Two rami	20	,,
Three rami	10	,,
Total	40	,,

(5) *Rami to* T2:

None	1	instance
One ramus	30	,,
Two rami	7	,,
Three rami	2	,,
Total	40	,,

These arose directly from the stellate in the twenty-seven instances where the second thoracic ganglion was incorporated in the fused mass. In twelve they came from a separate thoracic ganglion.

(6) *Ramus to that part of* T2 *which joins the brachial plexus.* This ramus was found in ten of the forty instances. It arose invariably from the stellate ganglion, irrespective of whether or not a second thoracic ganglion existed. It was a short thick ramus and joined the portion of the second thoracic nerve in such a manner that the latter often appeared to be its continuation. Indeed it was not always clear whether there existed

a true contribution from T2 to the brachial plexus, or merely a rather thick sympathetic ramus passing up to the first thoracic nerve.

Upper thoracic ganglia (T2–T7). The third to the seventh thoracic ganglia, and the second thoracic when separate, presented a uniform pattern (*b* in Fig. 1). Each ganglion was small and placed opposite the lower border of its corresponding vertebral body or more often the succeeding intervertebral disc. Fusion of two adjacent ganglia or duplication of a ganglion occurred rarely. Each ganglion was connected simply by one or more rami with its corresponding spinal nerve. A single ramus was the rule. Individual variations are listed in Table 1.

Lower thoracic ganglia (T8–T11). The lower thoracic ganglia represented a transition from the simple form of the higher thoracic ganglia to the more complex arrangement in the upper lumbar region. Slightly larger than the preceding ganglia, they are placed opposite the corresponding intervertebral disc or the next lower vertebral body. The relative position of the ganglion to the corresponding nerve was therefore lower and the rami consequently became more oblique (*c* in Fig. 1). Fusion and duplication were common. Each ganglion was connected, usually by two rami, with its corresponding spinal nerve, and in addition in some instances by a ramus with the spinal nerve of the next lower segment. This increasing tendency of a ganglion to connect with two spinal nerves instead of one was an almost constant feature of the eleventh thoracic ganglion and was the standard pattern in the upper lumbar region. Individual variations are summarized in Table 1.

Upper lumbar ganglia (L1–L4). These ganglia were the largest in the entire chain and were placed opposite the bodies of the corresponding vertebrae. Some form of fusion or of duplication was almost the rule in some part of this region. Each ganglion was connected by one or more transverse rami with its corresponding spinal nerve, and by an oblique ramus with the spinal nerve preceding, in some instances with a spinal nerve two segments higher (*d* in Fig. 1). The oblique ramus left the spinal nerve where it was entering into the formation of the lumbar plexus, therefore at a point unusually distal as compared with other levels. Zuckerman (1938) has noted this particularly in relation to the genito-femoral nerve. As the oblique connexions proved subsequently to be white rami, preganglionic fibres must travel considerable distances in the spinal nerve before emerging in the white ramus (*b* in Fig. 3). Often a ramus bifurcated so as to join two spinal nerves and conversely, two rami, each from a separate ganglion, frequently united to pass to one spinal nerve. The individual variations encountered were many, as will be seen from Table 1. Where a ganglion was connected with only one nerve, the other nerve usually received a ramus directly from the sympathetic cord either above or below the ganglion. In three instances the second lumbar ganglion was represented only by a small mass situated along the course of the grey ramus to L2 (*e* in Fig. 1).

Lower lumbar, sacral and coccygeal ganglia (L5–Cocc.). The form and connexions of these lower ganglia demonstrated a gradual 'return' to the pattern observed in the upper thoracic region, namely a smaller ganglion connected by one descending ramus, rarely two, with its corresponding nerve (*f* in Fig. 1). Duplication was rare, but absence of a ganglion, particularly in the lower sacral region, was common. There was always some form of fusion of the terminal parts of the sympathetic chains of each side, over the lower sacrum or upper coccyx, but sometimes this mid-line fusion occurred higher, once as high as the fifth lumbar. Individual variations are listed in

Table 1. *Analysis of the arrangement and connexion of the thoracic, lumbar, and sacral ganglia in forty dissections (rhesus monkey). The figures in heavy type represent the frequency of a double ramus*

	Anomalies						Common arrangements										
							Position			Rami communicantes							
										With one spinal nerve		With two spinal nerves		With three spinal nerves			
Ganglia	Fusion	Absence	Duplication	Total examples	Total examples	Size	Corresponding vertebral body	Intervertebral disc	Vertebral body below	Corresponding	One higher	Corresponding + one higher	Corresponding + one lower	Corresponding + one higher + one lower	Corresponding + two higher	None
3rd T	None	None	None	0	40	Small	8	32	—	39 **1**	—	—	—	—	—	—
4th T	None	None	None	0	40	Small	6	34	—	36 **4**	—	—	—	—	—	—
5th T	None	None	None	0	40	Small	7	32	1	31 **9**	—	—	—	—	—	—
6th T	None	None	None	0	40	Small	7	26	7	25 **14**	—	—	1	—	—	—
7th T	None	None	2	2	38	Small	7	20	11	25 **13**	—	—	—	—	—	—
8th T	1 (with 9th T)	None	None	1	39	Small	7	13	19	17 **20**	—	—	2	—	—	—
9th T	1 (with 8th T)	None	4	5	35	Small	5	15	15	8 **20**	—	—	7	—	—	—
10th T	None	None	6	6	34	Medium	4	14	16	7 **17**	—	—	10	—	—	—
11th T	None	None	4	4	36	Medium	—	—	36	2 **1**	—	—	33	—	—	—
1st L	1 (with 2nd L)	None	2	3	37	Medium	37	—	—	— —	1	35	—	1	—	—
2nd L	4 (1 with L1, 3 with L3)	2	1	7+3 ex. intermed. gang.	30	Medium	30	—	—	5 —	4	21	—	—	—	—
3rd L	5 (3 with L2, 2 with L4)	None	1	6	34	Large	34	—	—	3 —	2	27	—	2	—	—
4th L	1 (with 3rd L)	None	10	11	29	Large	29	—	—	— **1**	1	27	—	—	—	—
5th L	2 (midline fusion, 1 animal)	None	2	4	36	Large	36	—	—	17 **5**	—	9	—	1	4	—
6th L	None	2	None	2	38	Medium	38	—	—	30 **5**	—	—	3	—	—	—
7th L	4 (with 1st S)	2	1	7	33	Medium	33	—	—	17 **9**	—	—	4	3	—	—
1st S	6 (4 with L7, 2 in midline)	8	None	14	26	Small	26	—	—	18 **1**	—	2	—	—	—	5
2nd S	6 (midline fusion, 3 animals)	9	None	15	25	Very small	25	—	—	21 —	—	—	—	—	—	4
3rd S	24 (midline fusion, 12 animals)	1	None	25	15	Very small	15	—	—	12 —	—	—	—	—	—	3

Table 1. In one monkey with 13 ribs there was an extra thoraco-lumbar vertebra, which was called the eighth lumbar. Thus in two dissections (right and left sides) there was an eighth lumbar ganglion, with connexions on the right side with L7, L8 and S1, and on the left with L8 and S1 spinal nerves. On the right side of this animal the seventh lumbar ganglion was absent, and it is possible that the ganglion lying on the body of the eighth lumbar vertebra might have been a fused seventh and eighth lumbar. The coccygeal ganglia, minute in size, single or multiple, were almost invariably fused in the mid-line on the ventral surface of the coccyx, and gave a fine ramus on each side to the coccygeal nerves. The rami were extremely difficult to trace and variations observed were uncertain; for this reason they have been omitted from Table 1.

From the interganglionic parts of the sympathetic chain below the eighth thoracic level, rami were found joining the spinal nerves. They occurred most frequently between the eleventh thoracic and the third lumbar levels. In the sacral region such rami were associated with a missing ganglion, but elsewhere they were generally additional to the usual ganglionic rami, which might in such a case be reduced in number. The percentage frequency with which each spinal nerve received one or more rami communicantes is listed in Table 2.

Table 2. *Percentage frequency (nearest whole number) with which each spinal nerve receives one or more communicantes (rhesus monkey)*

Spinal nerve	Rami communicantes							
	None	One	Two	Three	Four	Five	Six	Seven
T1	—	28	45	27	—	—	—	—
T2	—	55	40	5	—	—	—	—
T3	—	98	2	—	—	—	—	—
T4	—	90	10	—	—	—	—	—
T5	—	78	22	—	—	—	—	—
T6	—	63	37	—	—	—	—	—
T7	—	63	35	2	—	—	—	—
T8	—	43	57	—	—	—	—	—
T9	—	18	75	7	—	—	—	—
T10	—	8	75	12	5	—	—	—
T11	3	20	67	10	—	—	—	—
T12	—	5	75	20	—	—	—	—
L1	—	8	55	35	2	—	—	—
L2	—	3	47	37	10	3	—	—
L3	—	—	25	35	28	8	2	2
L4	—	28	52	20	—	—	—	—
L5	—	73	27	—	—	—	—	—
L6	—	65	30	5	—	—	—	—
L7	—	40	55	3	2	—	—	—
S1	3	85	10	2	—	—	—	—
S2	13	77	10	—	—	—	—	—
S3	13	87	—	—	—	—	—	—
Cocc. 1	18	82	—	—	—	—	—	—

CONSTITUTION OF THE RAMI

It became evident from the histological studies of the rami that a simple classification into grey and white could not be followed. Instead, four general types came to be recognized (Fig. 2) and these have been designated as follows:

Type 1 consisted of myelinated fibres of all sizes, ranging up to 15 μ in diameter. The small fibres (3 μ and less) by no means dominated the picture. There was a fairly equal distribution of the various size groups, scattered irregularly throughout the

ramus, giving an appearance of lack of uniformity. This is evidently the white ramus as described by Gaskell and Langley. It was very distinct when present, and was sharply differentiated from the other types.

Type 2A consisted almost entirely of non-myelinated fibres, with often a fine scattering of small medullated fibres (under 3 μ in diameter) totalling not more than fifty odd in any single ramus. They were 'sprinkled' fairly uniformly throughout the section. Occasionally, and this was particularly true of the cervical rami, there was

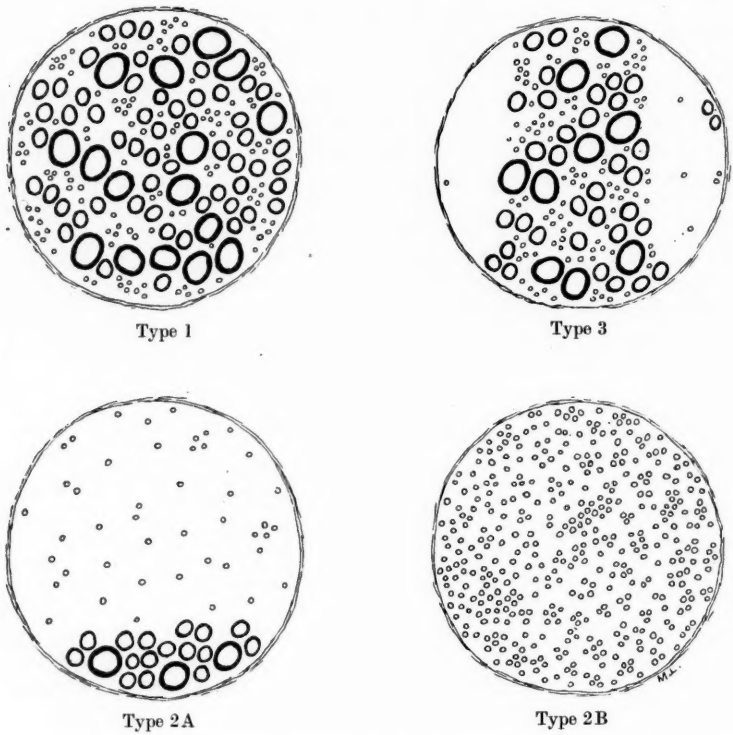

Fig. 2. Four types of rami communicantes in the rhesus monkey. Type 1 represents the white ramus; types 2A and 2B variants of the grey ramus; type 3 the mixed ramus.

within the nerve bundle a small group of ten to twenty large and medium myelinated fibres (10 to 5 μ in diameter). Type 2A has been regarded here as a characteristic grey ramus. As a type it merged imperceptibly into the next.

Type 2B was heavily 'peppered' throughout with small medullated fibres under 3 μ in diameter. The total number of such fibres per ramus amounted to several hundred. As in Type 2A, and again in the cervical rami, there was sometimes a small group of ten to twenty large and medium myelinated fibres (10 to 5 μ in diameter). There was no sharp distinction between the two types (2A and 2B) and they have been considered here as representing different varieties of grey rami.

Type 3 was clearly a mixed ramus, one part containing myelinated fibres of all sizes and having the typical appearance of type 1, and the rest resembling the grey ramus of type 2A. The two parts were together in the same nerve bundle, and did not represent two rami running in apposition.

Types 1 and 3 proved to be restricted to the thoraco-lumbar region which, in the twenty sides recorded here, was limited by the following boundaries:

Upper level:	T1 ...	In 8 instances
	T2 ...	In 12 instances
Lower level:	L3 ...	In 16 instances
	L4 ...	In 4 instances

This corresponds closely to the outflow of small medullated fibres in ventral spinal roots. The mixed rami (type 3) occurred chiefly in the upper thoracic region, where a single ramus was the rule. The grey rami were present everywhere, but type 2B was found characteristically in the rami of C8 and T1 and of L6 and L7, which often contained several hundreds of small myelinated fibres under 3 μ in diameter.

The upper cervical rami connecting the superior cervical ganglion with C1, C2 and C3 spinal nerves all belonged to type 2A. In the lower cervical region type 2B predominated. Occasionally there was a ramus composed principally of large and medium sized myelinated fibres (10 to 5 μ in diameter) with a small adjacent bundle characteristic of type 2A. Since the small myelinated fibres (3 μ and less in diameter) are missing, it could not be interpreted as a white ramus. The large myelinated fibres of this ramus are apparently somatic nerves to the prevertebral muscles running alongside a normal grey ramus. Wrete's embryological studies (1934–5) show this arrangement and we have confirmed it by dissection.

The first thoracic nerve was joined by a proximal and a distal ramus, and occasionally by a third, intermediate ramus. Contrary to the arrangement represented in standard diagrams, the distal and not the proximal branch was found to be the white ramus (type 1), and the others were grey (*a* in Fig. 3). This fact needs emphasizing for, although the arrangement is clearly stated by Langley (1896), Müller (1909), and Kuntz (1934), it has been largely overlooked in experimental and surgical literature.

In like manner, where the second thoracic nerve was connected by two rami, the distal of the two proved to be the white ramus. Where only one ramus joined T2 it was generally of the mixed (type 3) variety, and this was the arrangement in the succeeding five thoracic nerves (T3–T7) where each was joined usually by only one ramus. A typical nerve of the lower thoracic region (T8–T12), on the other hand, received two rami, one belonging to type 1, and the other to type 2A. A nerve of the upper lumbar region (L1–L4) was characteristically joined by two or more rami from different levels of the sympathetic cord. The upper *transverse* connexions were invariably the grey rami (type 2A) and the lower *oblique* connexions of types 1 or 3, as Botár (1932) has already shown. The rami of the lower lumbar nerves usually belonged to type 2B and were rich in small medullated fibres. In the sacral and coccygeal regions type 2A predominated, and the number of large and medium medullated fibres was negligible.

DISCUSSION

If the interpretation offered here of the four types of rami be correct, the definition of the thoraco-lumbar outflow in the monkey is essentially the same as that obtained from examination of the ventral spinal roots. It was not possible to study the constitution of the rami in the same series of animals that had been used for the ventral root investigation, but in the few examples in which both rami and spinal roots were examined the findings agreed exactly. At the upper boundary no white ramus was seen above T1 spinal nerve, and at the lower boundary none below L4. The lowest white ramus, however, ran from the third or fourth lumbar spinal nerve to join the sympathetic chain at, or below, the fifth lumbar ganglion. It would be necessary, then,

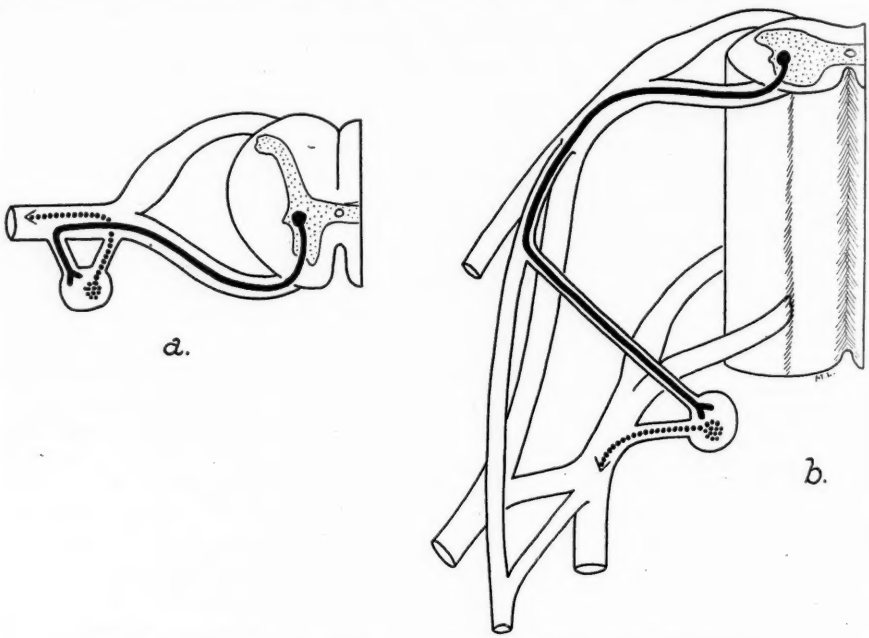

Fig. 3. Diagram of the course of the preganglionic fibre from (*a*) the first thoracic, and (*b*) an upper lumbar spinal nerve.

to divide the chain below this ganglion in order to cut all preganglionic fibres to the lower limb. Zuckerman (1938) mentions a single example where the lowest white ramus in the monkey passed out with L5 spinal nerve. It seems likely that some of the reports of an upward extension of the thoraco-lumbar outflow to C8, and even higher, are based on a misinterpretation of those rami found occasionally in the lower cervical region, composed chiefly of large and medium sized myelinated fibres and which we consider to be branches of somatic nerves to the prevertebral muscles.

The present investigation offers no support to the contention of Kiss & Mihálik (1929) that the rami cannot be differentiated histologically. It is true that the definition of what constitutes a white ramus may vary, but there can be no difficulty in dis-

tinguishing between types 1 and 3 on the one hand and types 2A and 2B on the other. There is a sufficiently sharp line of demarcation between the two groups to justify Gaskell's original subdivision into 'white' and 'grey' rami.

The white rami (type 1) are filled with medullated fibres of all sizes and, according to Ranson & Billingsley (1918), with unmyelinated fibres. Probably the larger proportion of the small medullated fibres (3 μ and less) are preganglionic, for they undergo degeneration when the ventral spinal nerve roots are cut and degenerated. The rest of the small medullated fibres, together with the medium and large medullated, and possibly also the unmyelinated fibres, are apparently afferent in nature, for they degenerate only when the dorsal roots are divided distal to the spinal ganglia and time allowed for degeneration (Ranson & Billingsley, 1918; Kuntz & Farnsworth, 1931).

The great number of medium and large medullated fibres in the white rami is very striking, particularly in the lower thoracic region, where there occurs the greater influx of afferent fibres from the splanchnic area. Indeed, the medium and large sized fibres together far overshadow the small medullated components, so that the rami seem to define a thoraco-lumbar inflow as much as an outflow.

The presence of myelinated fibres in grey rami has long been recognized (Langley, 1896), but their occurrence in such great numbers has been underestimated. We are not here referring to the groups of ten to twenty large and medium sized fibres which may occur in grey rami, for these disappear for the most part when the dorsal roots are cut and degenerated distal to the spinal ganglia (Kuntz & Farnsworth, 1931), and are therefore generally considered to be afferent. What interpretation, however, is to be placed on the small medullated fibres which are scattered throughout a grey ramus, often, as in type 2B, in great numbers? Many persist after section and degeneration of both spinal roots distal to the spinal ganglion (Langley, 1896; Nevin, 1930; Kuntz & Farnsworth, 1931) and are therefore presumably medullated postganglionic fibres. We have been able to confirm this in the monkey (unpublished experimental data), but it is not yet clear how many fibres do degenerate following such a procedure. They are considerable in number. Langley (1896) and Wrete (1935) have suggested that some of these small myelinated fibres in grey rami may be preganglionic fibres passing to ganglion cells more peripherally placed, along the course of the grey rami ('intermediary' ganglia) or even within the spinal nerves. Examples of such intermediary ganglia, clearly recognizable grossly, have been reported here, but it seems that they may be much commoner than has been generally supposed, particularly when a microscopical search is made for them. Langley (1896) called attention to their existence, and subsequent reports have been made by Romankevic (1930) and Gruss (1932). Kuntz (1934) explains their presence as due to cells which either failed to reach the primordia of the ganglia of the sympathetic trunk during embryological development or became displaced from them. Wrete (1935) has described their frequency and distribution, and finds them located mainly in two general regions, cervical and lumbar. It is significant that the grey rami of these regions are particularly rich in small medullated fibres.

Attention has been drawn to the interconnexions between the sympathetic chains of each side, as they offer pathways through which an organ may be innervated contralaterally. The possibility of a bilateral innervation to the limbs has been weighed in attempts to explain the fact that unilateral thoracic or lumbar sympathectomy produces, in addition to its effect on the ipsilateral limb, a vasomotor change in the

corresponding contralateral limb (Simmons & Sheehan, 1939). In the monkey, however, no transverse connexions existed above the fourth lumbar level. Furthermore, no action potentials could be picked up in the sciatic nerve following stimulation of the ventral roots of the lower thoracic and upper lumbar segments on the contralateral side (Sheehan & Marazzi, 1941). The transverse connexions do not therefore appear to be concerned significantly in the innervation of the limbs.

The difference in the gross anatomy of the ganglia at various levels is most conspicuous. In the upper thoracic, lower lumbar and sacral regions the spinal nerve is almost invariably connected to the ganglion of the same segment by one or several rami. This simple segmental arrangement does not exist in the cervical or in the lower thoracic and upper lumbar levels, where a ganglion sends rami to two or more spinal nerves. In addition, it is in these regions that irregularities of the ganglia in the form of fusion, duplication or absence are most frequent. The primordia of the ganglia of the lower thoracic and upper lumbar levels presumably divide, the caudal portion of each fusing with the upper part of the next succeeding primordial mass. A permanent ganglion in these areas is then the result of fusion of portions of two succeeding ganglia, and their rami obviously join two or several spinal nerves (Fig. 4). Splitting and secondary fusion may explain the frequent anomalies of the ganglia within this region.

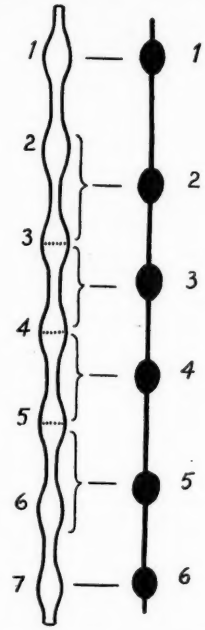

Fig. 4. Schematic representation of the development of the ganglionic chain from the primordial cell column in the lower thoracic and upper lumbar regions.

SUMMARY

1. A study of the connexions and constitution of rami communicantes in the macaque reveals four general types. One (type 1) is filled with myelinated fibres of all sizes and has been interpreted as the white ramus. Two other types (types 2A and 2B) are composed of unmyelinated fibres with differing amounts of myelinated fibres, distributed throughout the ramus. They represent variations of the grey ramus. The fourth type (type 3) is mixed, with areas of 'grey' and 'white' in the same nerve bundle.

2. The white and mixed rami do not extend beyond the limits of the thoracolumbar outflow as outlined in the previous study of the ventral spinal roots—namely T1/T2–L3/L4, rarely L5.

3. In the lower cervical region a branch of the spinal nerve to the prevertebral muscles may run with a grey ramus for the greater part of its course, and may be mistaken for a white ramus to a lower cervical nerve.

4. The small myelinated fibres (3μ or less) in grey rami may number several hundred, particularly in those of C8 and T1 and of L6 and L7 spinal nerves. Many of these fibres are myelinated postganglionic, but some may be preganglionic, reaching intermediary ganglia placed along the course of the ramus or within the spinal nerve.

5. The distal, and not the proximal, ramus to the first thoracic nerve is invariably the white ramus.

6. In the upper thoracic, lower lumbar, and sacral regions a ganglion is connected with its corresponding spinal nerve, usually by only one ramus, which in the upper thoracic region is of the mixed type. In the lower thoracic and upper lumbar regions the ganglia are larger and more irregular, and each is connected with two or more spinal nerves, by a transverse (grey) ramus with its corresponding spinal nerve, and an oblique (white) ramus descending from the spinal nerve one or two segments higher.

7. Some form of transverse connexion between the sympathetic chains of each side was found in every dissection, but never above the level of the fourth lumbar vertebra. A double sympathetic chain occurred only in the lumbar region and never extended for more than the distance between two adjacent ganglia.

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STORAGE OF TRYPAN BLUE IN THE HEART VALVES OF THE RABBIT

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In recent investigations (Harper, 1938, 1940) dealing with the structure of mammalian heart valves it was shown that the subendothelial zone of the cusps contains several cell types. Employing the method of intravital staining, this study has been continued, and the present paper contains an account of observations on the reaction of the cells in normal rabbit valves to the subcutaneous injection of trypan blue. The results obtained led to further study of the cellular reaction produced by particulate damage to rabbit valves caused by injection of aleuronate, and data relative to this and to the formation of new blood vessels which occurs within the subendothelium under these conditions (Harper, 1938) are also presented.

METHODS

The experiments were designed to test the early and later effects of trypan blue in moderate concentration and the effects of gradually increasing the dose. The dye was administered subcutaneously in a 2 % solution in distilled water and the animals were killed at various intervals after stopping the injection. In the second part of the investigation particulate damage to the valves was effected by means of the pseudo-crystalline substance aleuronate in normal saline suspension, the animals receiving in addition subcutaneous injections of trypan blue at various intervals following the administration of the aleuronate. The method employed for the preparation and use of the aleuronate suspension is described in a former paper (Harper, 1938). Before use the trypan blue solution was filtered and then sterilized by gentle boiling. The amounts of the dye and aleuronate suspension used, the frequency of the injections, and the times when the animals were killed are given in Table 1. After being opened to expose the valves, the hearts were fixed in Heidenhain's 'Susa' or a formalin acetic mixture (5 c.c. glacial acetic acid in 100 c.c. 10 % formalin), both of which preserved the trypan blue colour well.

STRUCTURE OF NORMAL ATRIO-VENTRICULAR VALVES IN THE RABBIT. GENERAL REACTION TO TRYPAN BLUE

The atrial endothelium consists of a single layer of rounded cells with non-granular protoplasm and moderately dense nuclei. Trypan blue was not observed in the cells of this layer in any of the experiments. In the subendothelium two cell types, the fibroblast and the macrophage, can be readily distinguished by ordinary staining methods. The fixed fibroblast occurs usually adjacent to the surface of the collagenous bundles in this zone; its cytoplasm is clear and the nucleus is long and oval and commonly contains two nucleoli. This type of cell is not the commonest connective tissue element in the subendothelium, and it accumulates no trypan blue even in high concentration. As in human valves, somewhat smaller cells with the same general appearance as the fixed fibroblast, are abundant, and in the proximal two-thirds of the cusps they outnumber the latter. The cytoplasm of these cells frequently contains

minute dust-like particles, and in contrast to the fixed type they are capable of segregating trypan blue on moderately intense stimulation. The relative number of these cells, their affinity to the dye, and their reactions in aleuronate-damaged cusps suggests that they are not common fibroblasts but rather undifferentiated connective tissue cells which under the action of certain stimuli, e.g. normal wear and tear of the cusps, inflammation, etc., probably undergo progressive development to furnish other cell types.

Macrophages are abundant throughout the entire subendothelium. They are as numerous as the fixed fibroblasts, and in the intermediate portions of the cusps they are frequently assembled in small rounded or elongated groups near the atrial surface. Their form varies from a rounded to an elongated spindle shape, sometimes with bluntly pointed processes. In contrast to the fibroblast, the nucleus is relatively small, frequently irregular or in two parts and without large nucleoli. Following the injection of trypan blue even in low concentration into the living animal the macrophages accumulated blue particles in their cytoplasm almost immediately, and when repeated injections were made the dye inclusions became more numerous and larger. The method thus allows a precise identification of these cells. The selective storing of trypan blue by cells of this type is so pronounced that the whole valve becomes blue to the naked eye.

Collagenous fibres are abundant in the main mass of the subendothelium, and elastic fibres are mostly limited to its deeper parts adjacent to the zona fibrosa of the cusp. The latter consists of a basophil collagenous substance containing only a few rounded cells and some fine elastic fibres. Relatively few cells of the normal zona fibrosa are capable of segregating trypan blue in moderate concentration. Direct continuity exists between the 'fibrosa' and the central fibrous core of the chordae tendineae at the sites of their insertion.

In the narrow ventricular subendothelium the fixed fibroblasts outnumber the undifferentiated connective tissue cells, and macrophages are relatively fewer in number than on the atrial side. The endothelium on this aspect of the cusp is not prolonged on to the chordae tendineae, but the latter are surrounded throughout their entire length by a sleeve composed of a layer of two to three cells in thickness continuous with the ventricular subendothelium above and the corresponding layer on the papillary muscles below. The majority of the cells constituting the sleeve are capable of segregating trypan blue on intense stimulation. Their reaction to the dye will be described and discussed at a later stage.

Before detailing the degree of storage of trypan blue by the various cells, it may be pointed out that the atrio-ventricular valve cusps in the rabbit, like those of man, are almost completely non-vascular. Very short blood vascular extensions composed of terminal capillary loops associated with myocardial prolongations of similar extent are, however, present in the proximal parts of the cusps, but these rarely extend for more than a distance of 1 mm. from the attached margin, so that approximately seven- to eight-tenths of the entire valve cusp is devoid of blood vessels (Harper, 1938).

DEGREE OF STORAGE OF TRYPAN BLUE

It is well known that following injections of trypan blue into the tissues the dye is taken up first by the macrophages. These cells possess in pre-eminent degree the capacity for vital staining, and blue granules appear in their cytoplasm even when

relatively weak solutions of the dye are employed. In the heart valves of the rabbit, as in other tissues, the segregation of trypan blue by the macrophages is rapid and the degree of storage depends largely on the amount injected.

In R 1, killed approximately 12 hr. after a single injection of 2·5 c.c. of the dye, stained macrophages were observed throughout the whole extent of the subendothelium of the atrio-ventricular cusps. Their distribution with regard to surface area and depth was fairly uniform in the proximal two-thirds of the cusps, but towards the free margin they were more sparsely distributed. The segregated dye particles were rather pale in colour and evenly distributed throughout the cytoplasm of these cells (Pl. 1, fig. 1).

After injections on two successive days of the same amount of trypan blue, and delaying the time of killing (R 2 and R 3), the intensity of the staining increased and the form of the macrophages became more clearly defined. Their relative number 48 hr. after the last injection was not, however, appreciably increased. The accumulated dye appeared in the form of dark blue particles occupying the greater part of the cell and so packed as occasionally to obscure the nucleus completely (Pl. 1, fig. 2). In form the cells are rounded and commonly have blunt or pointed processes. They vary in size, but all possess the power of segregating trypan blue rapidly and completely

Table 1

Rabbit	Daily dose of trypan blue c.c.	No. of injections	Time between last injection and killing	Rabbit	Daily dose of trypan blue c.c.	No. of injections	Time between last injection and killing
1	2·5	1	12 hr.	6	7	2	2 days
2	2·5	2	2 days	7	7	2	5 days
3	2·5	2	10 days	8	10	2	2 days
4	5	2	2 days	9	10	2	10 days
5	5	2	10 days				

R 10, R 11 and R 12 each received three daily doses of 5 c.c. each of aleuronate suspension followed approximately 14 hr. after the last injection by two daily doses of 5 c.c. each of trypan blue. R 10 was killed 12 hr. after the last injection of the dye and R 11 and R 12 at intervals of 7 and 29 days respectively.

throughout their cytoplasm. In R 3, which was killed 10 days after the last injection, there was again no appreciable increase in the number of macrophages present, but slight fading of the dye had occurred together with disruption of some dye-containing cells in the superficial parts of the subendothelium.

When the initial dose was doubled and the animal, R 4, killed 2 days after the last injection, the macrophages became more abundant in both the mitral and tricuspid valves, and the intensity of the staining more marked especially in regions subjacent to the area of contact of the cusps. In addition, a few smaller undifferentiated connective tissue cells in the proximal parts of the cusps acquired small particles of the dye. Employing this concentration, which may be regarded as moderate, there is thus little evidence of any cell in the subendothelium other than the frank macrophage, containing dye particles, even with delay in killing the animal (R 5). In view of this and of the effect produced by increasing the dosage it is considered that this stage represents the normal histiocytic content of the cusps. The atrio-ventricular valves of R 5, which was killed 10 days after the last injection, exhibited less intense staining of the macrophages, several of which had liberated their dye particles. No vital staining of the sleeve cells around the fibrous core of chordae tendineae appeared under moderate stimulation.

With increasing dosage (R 6), the number of dye-absorbing cells increased rapidly. The majority of them were again histiocytic in nature, many being at different stages of development, but in addition the dye now clearly revealed other cells throughout most of the subendothelium (Pl. 1, fig. 3). They were smaller in size than the typical macrophages and the trypan blue particles in their cytoplasm showed a tendency to be arranged at the poles of the nucleus. They were most abundant in the proximal portion of the valve adjacent to the myocardial extensions, where, in fact, cells of similar type were first observed when a smaller dosage of the dye was employed. When the time of killing the animal was delayed (R 7), the dye inclusions became more numerous and deepened in colour in both the macrophages and the undifferentiated cells. In the latter the bipolar arrangement of the particles largely disappeared, particularly in the regions immediately subjacent to the area of contact of the cusps, and many of these cells assumed a form almost identical with the smaller macrophages. Differentiation of the two cell types, more apparent at an earlier stage with less intense stimulation, was thus no longer possible. Compared with the typical macrophage the dye particles in the undifferentiated connective tissue cells are relatively smaller, and the reduction in size of the cells appears to be chiefly in the cytoplasm. Their presence as revealed by increasing concentrations of the dye indicates that there may be a continuous new formation of macrophages from this source.

Employing trypan blue in the above concentration, the storage of the dye within the cells of the subendothelium was fairly intense and a mild cellular reaction was produced. The cellular picture, therefore, cannot be regarded as normal. Further, the reaction was not confined to the subendothelium, for the atrial endothelial cells were themselves altered. They became swollen and rather oedematous in appearance, stained feebly, and in places tended to disappear altogether.

The sleeve cells surrounding the fibrous core of chordae tendineae in R 6 reacted feebly to the dye and exhibited all the appearances of undifferentiated connective tissue cells. In R 7, in which the time of killing was delayed, this effect became more pronounced and there was evidence of some overlap, or perhaps syncytial fusion of these cells. The possible significance of the appearances obtained in this region will be discussed later.

Following larger doses of trypan blue, the atrio-ventricular valves of the animals R 8 and R 9 became further loaded with dye-absorbing cells. These were mainly histiocytic in nature, and in this overstained condition it was impossible to decide whether certain cells were undifferentiated connective tissue cells or relatively inactive macrophages. The general appearance of the vitally stained subendothelium in these animals was very similar to that obtained after the administration of trypan blue following early aleuronate damage (see Pl. 2, fig. 4). There was no evidence of proliferation of capillary blood vessels as a result of the cellular reaction produced in these experiments.

It would appear that with moderately intense stimulation by trypan blue the maximum intensity of intracellular staining is reached in about 48 hr. This persists with no appreciable diminished activity for some 3–5 days, after which gradual fading occurs. Intercellular dye masses, commonly of a narrow elongated form, were present in cases of moderately intense stimulation, particularly when the animal was killed some time after the last injection. They occurred also at earlier periods following larger doses, and it is possible that they may have been liberated from cells loaded

with the dye or as a result of their disintegration. It may be recorded here that the hearts of the experimental animals became stained in greater or less degree, and this was due mainly to the presence of vitally stained cells, principally macrophages, in the intermuscular septa.

REACTION OF THE RABBIT TRICUSPID TO TRYPAN BLUE FOLLOWING THE INTRAVENOUS INJECTION OF ALEURONATE

It has been shown that following mechanical damage to the atrial surface of the tricuspid valve by means of the intravenous injection of aleuronate a considerable cellular reaction occurs within its subendothelium (Harper, 1938). Oedematous swelling and partial desquamation of the atrial endothelium and an increased number of mitotic figures in the subjacent tissue can be observed at a comparatively early period—about 12 hr. after the initial injection. Within 24 hr. the subendothelial cells and particularly those beneath the area of contact of the cusps increase in number and in size. Swelling of the collagenous reticulum accompanies the cellular reaction.

To study the reaction of particular cells by intravital staining, trypan blue in moderate concentration was superimposed on the damaged valve approximately 14 hr. after the last injection of aleuronate suspension, and the animal, R 10, was killed 12 hr. later. Intense staining of the subendothelial cells resulted and the majority of them appeared to be histiocytic in nature (Pl. 2, fig. 4). The appearance of many of the smaller dye-containing cells, however, suggests that they are undifferentiated connective tissue cells which have probably been activated by the particulate damage to the cusps and are now much more receptive to the dye. The general appearance of the subendothelium resembles that produced by intense stimulation by trypan blue alone. But the cells are more intensely stained throughout the entire depth of the zone, and the intracellular dye particles are appreciably larger. On account of the intense cellular reaction elicited in this experiment larger doses of trypan blue were not employed. Clearly the dye had much more direct access to the subendothelial cells through the damaged atrial surface, and its segregation was so rapid and complete as to render identification of cell types almost impossible.

R 11 and R 12 each received the same initial dosage of aleuronate and trypan blue and were allowed to survive for 7 and 29 days respectively after the last injection of the dye. The appearance of the subendothelium of the tricuspid valve in R 11 is shown in Pl. 2, fig. 5. The dye-containing cells are chiefly macrophages, and there appears to have been a general migration of these towards the damaged atrial surface. This feature was specially marked along the area of contact of the cusps where, presumably, they are eliminated by desquamation or disruption. Their presence in this situation masks the repair processes which are beginning at this time. In R 12, which was killed approximately 1 month after the administration of trypan blue, the healing process was well advanced and connective tissue cells, among which a few dye-stained macrophages still persist, have formed a compact almost pallisade-like thickening on the atrial surface (Pl. 2, fig. 6).

As already pointed out, the cellular reaction following particulate damage to the tricuspid valve is accompanied by a new growth of capillaries into the subendothelium of its cusps. Employing the amounts of aleuronate cited in Table 1, signs of commencing proliferation of new blood vessels can be observed in 24 hr., although less

time is probably required for their formation after the initial dosage. The new capillaries arise as endothelial outgrowths from pre-existing vascular endothelium at the bases of the cusps and advance as blindly ending capillary sprouts. The original form of the vascular network is that of an indefinite capillary plexus, but as it continues to advance union of the vessels occurs. Simultaneously, many intervening capillaries retract and disappear and the adult plexus is relatively stable.

From the scanty material on which observations on the growing capillary plexus were possible it was found that dye-containing connective tissue cells of the fibroblast type were associated with the advancing capillaries, adhering to their outer walls. It was not always easy, however, to determine whether some of the cells actually lay in the capillary wall or just without it, and thus to decide whether one was dealing with a vitally stained endothelium or with adventitial cells destined to become an integral part of the vessel wall. The difficulty was further increased by the proximity of numerous macrophages at various stages of development. Further experimental work will be necessary before the precise role of these cells in the formation of the definitive vessel wall can be ascertained. It may be observed, however, that the form of many of the cells on, or near, the walls of the new blood vessels is in all respects similar to the extra-endothelial cells of Clark & Clark (1940) who described their association with growing capillary vessels seen in transparent chambers introduced into the ears of rabbits.

DISCUSSION

Vital staining methods which have demarcated clearly defined groups of cells in other parts of the animal body can be applied to a study of the subendothelium of the heart valves of the rabbit. When trypan blue is injected subcutaneously, typical macrophages and undifferentiated connective tissue cells in this zone present a characteristic appearance. The macrophages exhibit the property of accumulating the dye rapidly and in marked degree, while in the undifferentiated cell it is much less highly developed. Under more intense stimulation both types come to contain a large amount of the dye, and it becomes almost impossible to decide whether certain cells are fibroblasts or relatively inactive macrophages. The property of vital staining here, as in other connective tissues of the body, is, therefore, not a hard and fast attribute of certain cells but is rather an indication of a functional activity on the part of the cells at the time of examination. The number of fully formed dye-containing macrophages observed at any given time may be taken as an index of the activity, and it has been shown that this is modified under the influence of experimental conditions. In this connexion the relatively greater number of these cells normally present in regions subjacent to the area of contact (site of wear and tear) of the cusps is noteworthy.

Under normal conditions the majority of the macrophages are probably non-motile elements, rounded or amoeboid in form in structural adaptation to their cellular environment. When loaded with trypan blue they may liberate their dye particles, and there is evidence also that they are capable of migrating towards the atrial aspect of the cusps where they are eliminated. The latter feature was specially marked during the repair process following aleuronate damage. It may be presumed, therefore, that subendothelial macrophages under pathological conditions are capable of absorbing and possibly neutralizing soluble toxic substances and thus play an important role in the so-called local defence reaction. Storage of trypan blue may be regarded as a

local defence activity, and when loaded with dye particles these cells will become incapable or less capable of performing other functions.

Notwithstanding the non-vascular nature of valvular subendothelium, the receptivity of its cells, and especially of macrophages, to trypan blue is as well marked as in tissues well supplied with blood. Further, the toxic effect of the dye, as indicated by the cellular reaction produced in this region by increasing doses, occurs earlier and is more marked than in most tissues following similar doses. It is of interest to note that the experimental results detailed here tend to confirm the suggestions made by Dietrich (1926), Pfuhl (1929), Semsroth & Koch (1930), and Thomson (1935), concerning the detoxifying ability and sensitization of valve tissue in relation to the genesis of endocarditis.

The segregation of the dye by undifferentiated connective tissue cells following moderately intense stimulation is in its early stages quite characteristic and resembles that which occurs in other tissues (Evans & Scott, 1921; Cappell, 1929; King, 1938). When increasing amounts of the dye are presented, however, the majority of these cells rapidly acquire staining characters resembling those of macrophages, and recognition of the two types becomes difficult or impossible. Cappell (1929) has shown that cells exhibiting intermediate staining characters in subcutaneous areolar tissue are at first unstained, but they rapidly develop a latent tendency for vital staining and in a relatively short time become indistinguishable from the fully formed macrophage. The presence of intermediate cell types as revealed by increasing stimulation by trypan blue is suggestive of a new formation of macrophages from undifferentiated connective tissue cells of the subendothelium.

The resemblance of the dye-containing cells associated with the walls of new capillary blood vessels to the extra-endothelial cells observed by Clark & Clark (1940) on regenerating capillaries in the rabbit's ear has been pointed out. These authors described the approach of the cells to the outer walls of the growing vessels where they become attached and remain as adventitial cells. Later they undergo changes in both form and arrangement, and it was shown that most of the new muscle cells on regenerating arterioles are formed in this manner. It is suggested that the cells described here are similarly endowed with the potencies of embryonic mesenchymal cells and that by progressive development they furnish other cell types associated with, and taking part in, the formation of the new capillary plexus. The dye-receptive cells surrounding the fibrous core of chordae tendineae are probably of the same nature as the above. Chordae are normally non-vascular structures, but in human valvular endocarditis blood vessels have been observed extending along their entire length connecting the vasculature of papillary muscles to blood vessels of inflammatory origin in the cusps (Harper, 1938). Under these conditions the sleeve cells, which occur also in man, doubtless play a part in the establishment of new blood vessels at these sites.

Following experimental damage to the tricuspid valve, the subendothelial reaction as revealed by intravital staining was much more intense and extensive than was previously recorded (Harper, 1938). The increased staining property exhibited by macrophages, and to a less extent by undifferentiated connective tissue cells, when trypan blue was superimposed on damaged cusps, is presumably accelerated by an increased permeability of the valve tissue. It is interesting, however, to inquire further into the possible causes of the intense and rapid segregation of the dye under these conditions. The substance aleuronate which was used to produce particulate damage

to the cusps in these experiments is largely protein in nature, and there is a possibility that this substance was accumulated by the macrophages in particulate or soluble form prior to the injection of the dye. It is known, however, that cytoplasmic inclusions are rarely stained by acid dyes, but instances have been recorded, and these include protein granules in the epithelial cells of the intestine of young mice (Mollendorff, 1921). Stained particles of absorbed protein may thus account for the increase in number and size of the inclusions observed in the subendothelial cells under these conditions. The cusps of the mitral valve in animals to which aleuronate had been administered suffered no obvious endothelial damage, but more intense vital staining of the subendothelium indicated that in this situation, also, there was greater receptivity to the dye.

SUMMARY

1. Macrophages and undifferentiated connective tissue cells in the subendothelium of the heart valves of the rabbit present a characteristic appearance following the subcutaneous injection of trypan blue in moderate concentration.

2. Under more intense stimulation the dye particles in both types increase in number and size and their differentiation becomes difficult or impossible.

3. The increased staining property exhibited by undifferentiated connective tissue cells is suggestive of a continuous new formation of macrophages from this source.

4. Macrophages containing trypan blue particles may liberate their dye content, and there is evidence also that they migrate towards the atrial aspect of the cusps where they are eliminated.

5. Undifferentiated dye-containing cells are associated with the walls of new capillary blood vessels, and it is suggested that these cells are endowed with the potencies of embryonic mesenchymal cells furnishing other cell types taking part in the formation of the definitive plexus. Many of them also function in the repair process following particulate damage to the cusps.

6. The general receptivity of subendothelial cells to trypan blue is compared with that shown in other tissues.

7. The toxic effect of the dye on the cells is discussed.

The author's thanks are due to Prof. J. D. Boyd for helpful criticism.

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EXPLANATION OF PLATES 1 AND 2

All photomicrographs are those of longitudinal sections through the cusps and show the atrial endothelium and subendothelium only, except in the case of fig. 4, in which the zona fibrosa is visible.

Plate 1

Fig. 1. Anterior cusp of mitral valve of R 1, 12 hr. after a single injection of trypan blue. Note pale dye particles in the macrophages. van Gieson, ×450.

Fig. 2. Anterior cusp of mitral valve of R 2, 48 hr. after two injections of trypan blue. Note prominent dye inclusions in the macrophages. Carmalum (Kirkpatrick's modification), ×450.

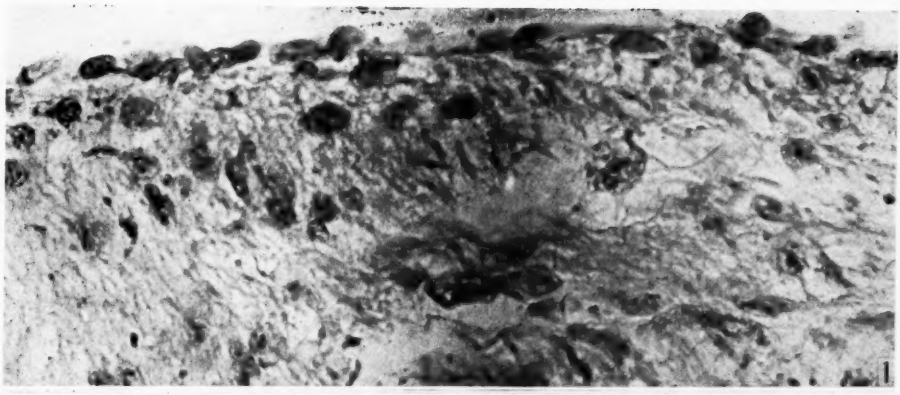
Fig. 3. Proximal part of anterior cusp of tricuspid valve of R 6, after moderately intense stimulation by trypan blue. Note increase in number of dye-absorbing cells (macrophages and undifferentiated connective tissue cells). van Gieson, ×450.

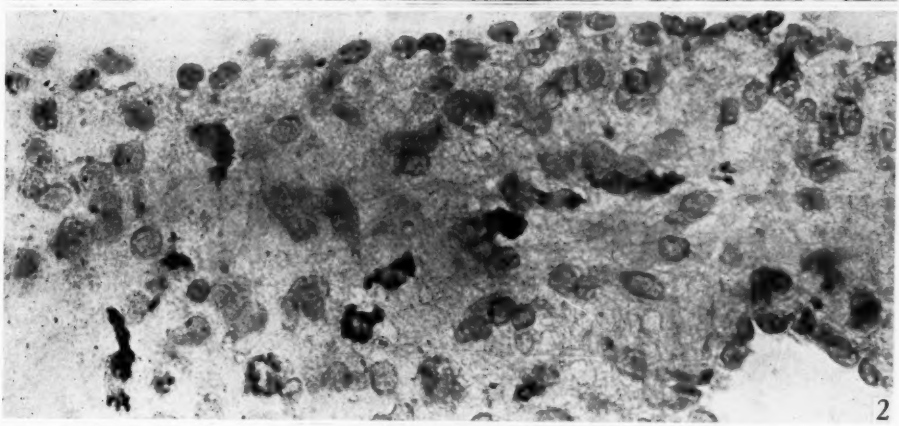
Plate 2

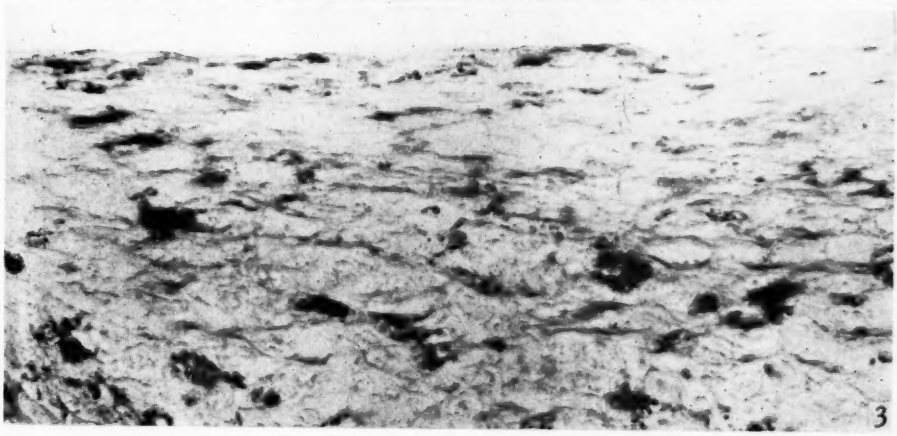
Fig. 4. Anterior cusp of a damaged tricuspid valve (R 10), 14 hr. after injection of trypan blue. Note intense vital staining of subendothelial cells and desquamation of atrial endothelium. *F*, zona fibrosa. van Gieson, ×400.

Fig. 5. Anterior cusp of a damaged tricuspid valve (R 11), 7 days after injection of trypan blue. Note dye-containing cells, chiefly macrophages, near atrial surface. van Gieson, ×450.

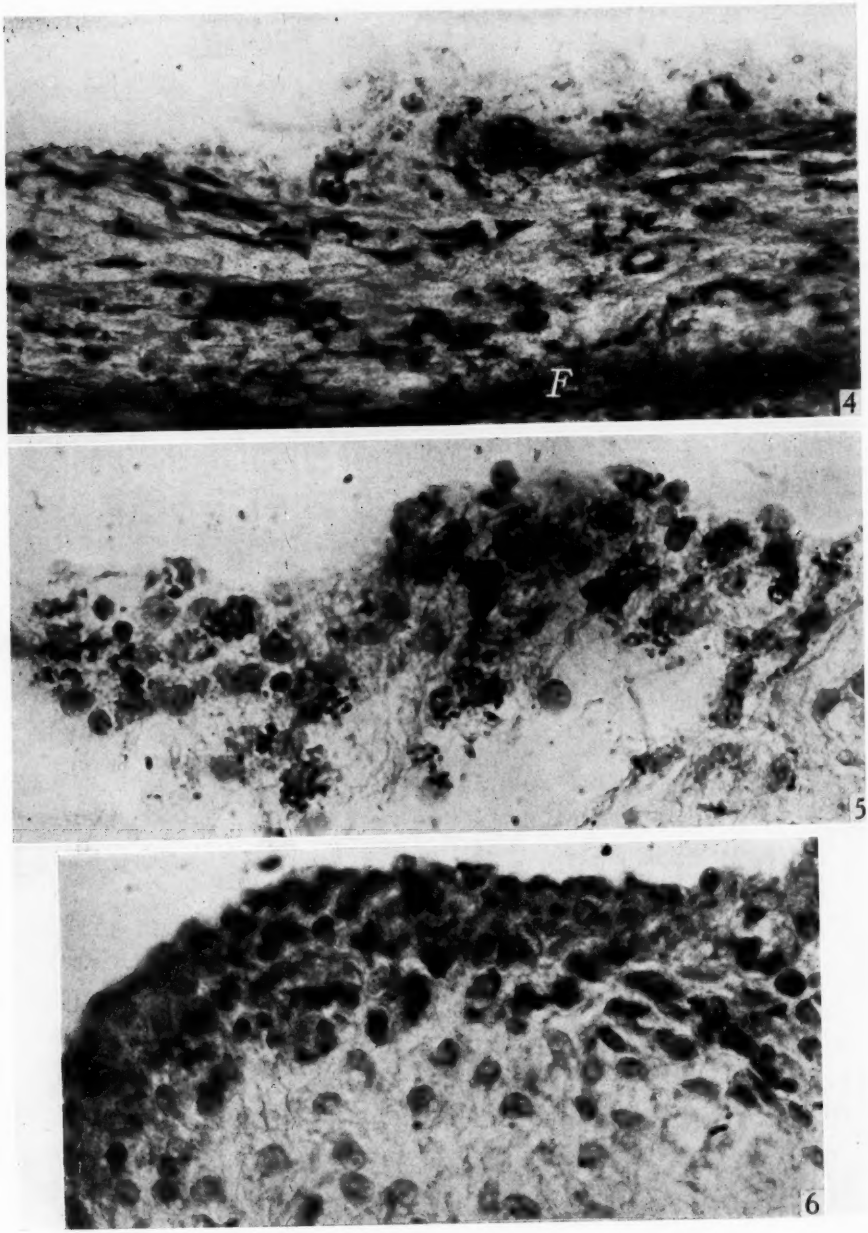
Fig. 6. Anterior cusp of a damaged tricuspid valve (R 12), 29 days after injection of trypan blue. Note compact pallisade-like thickening on atrial surface and some dye-containing macrophages. van Gieson, ×450.







HARPER—STORAGE OF TRYPAN BLUE IN HEART VALVES



HARPER—STORAGE OF TRYPAN BLUE IN HEART VALVES

LOCALIZATION OF NEWLY ADMINISTERED IODINE IN THE THYROID GLAND AS INDICATED BY RADIO-IODINE

By C. P. LEBLOND, *Department of Anatomy, McGill University, Montreal*

With the help of radio-iodine, it has been possible to show that in physiological conditions iodine is first incorporated in the thyroid gland as diiodotyrosine and from there is slowly transformed into thyroxine (Mann, Leblond & Warren, 1942). In order to determine the histological localization of the iodine entering the gland, sections of thyroid glands from animals given radio-iodine were placed on photographic plates. Since each accumulation of radio-iodine in the section affects the plate, it is possible to deduce the localization of the radio-iodine from a study of the pictures thus obtained. This 'autographic' method was first applied to a study of the localization of polonium (Lacassagne, Lattes & Lavedan, 1924, 1925). Later, Hamilton, Soley & Eichorn (1940) showed that after administration of radio-iodine normal thyroid tissue, but not thyroid cancer, acted on the photographic plate. Gorbman & Evans (1941), in tadpoles given radio-iodine at various stages of development, noted that sections from thyroids with more colloid impressed the plates more markedly.

TECHNIQUE

The isotope I^{130}, with a half-life of 12 hr., from the cyclotron of the University of Rochester, was used on dogs and rabbits. But more satisfactory results were obtained with the 8-day isotope I^{131} prepared at the Massachusetts Institute of Technology, used on guinea-pigs and rats. Since the radio-iodine is prepared from tellurium in the cyclotron, the number of radio-active atoms is relatively small and their quantity cannot be expressed in chemical terms. This minute amount of radio-iodine was used alone in the experiments with dogs. In the experiments with other animals, some ordinary chemical iodine (I^{127}) was added as a carrier in various proportions as indicated in Table 1. In all cases, the radio-iodine was given in a single injection.

Table 1

Animal species	Approximate animal weight kg.	Isotope	Dose of iodine μg. per kg. body weight	Autopsy time after injection hr.
Dog	20	I^{130}	Infinitesimal (no carrier)	½, 8 and 48
Rabbit*	3	I^{130}	5	6 and 12
Guinea-pig	0·1	I^{131}	30	6 and 12
Rat	0·1	I^{131}	100	24
	0·1	I^{131}	5000	24

* Kindly furnished by W. Mann.

At various time intervals after injection, the animals were killed and two small fragments of thyroid were taken: one for routine paraffin technique (Bouin, alcohol, xylol, paraffin). When the 12 hr. isotope was used, the technique was speeded up so that the sections were affixed to the slide from 6 to 8 hr. after autopsy: the slides were then passed through xylol, alcohol, 0·5 % celloidin and 70 % alcohol and finally dried. The other thyroid fragment was treated by a simplified freezing drying technique after

the Altmann-Gersch method, including freezing in acetone-dry ice mixture, drying in a Pyrex test-tube connected with a Hyvac pump and refrigerated at a temperature of −20° C. After drying was completed, some paraffin present in the test-tube was melted. The impregnation was immediate and sectioning could be rapidly carried out. The sections were affixed to the slide by rapidly stretching them over slides heated at 56° C. without using water.

The slides bearing the sections were then placed on the sensitive face of medium lantern slide plates. Difficulties produced by the adhesion of paraffin to the gelatine emulsion of the plate in the case of frozen dry sections could be overcome by interposing thin microfilms of collodion. The slides were left for several days in the dark on the lantern slide. Each accumulation of radio-iodine in the section affects the photographic plate, provided the contact between the plate and the section is quite close. The plate is developed and fixed in the usual manner. The section is then stained; either routine hematoxylin-eosin or Masson's trichrome were used. For comparison of the autograph with the histological section, photographic enlargements of both were made at the same magnification.

RESULTS

Sections of thyroid tissue reacted on the photographic plate markedly and in the same fashion in all animal species examined. Sections of kidney and liver, however, did not affect the photographic plate. Similarly, no reaction was produced by other tissues present in the thyroid sections, such as blood vessels (Pl. 1, figs. 1, 2), parathyroid gland (figs. 3, 4) and connective tissue (Pl. 1, figs. 1–6; Pl. 2, figs. 7, 8). These results confirm the strong affinity of thyroid tissue for iodine, already established by chemical analysis (bibliography in Leblond, 1942).

The reactions produced by the colloid accumulations and the groups of cells were then examined comparatively. It may be seen in Pl. 1, fig. 1 that a spot on the autograph corresponds to each colloid accumulation. In contrast, two well-defined groups of cells, probably resulting from sections through the walls of follicles, produce only a very slight impression on the plate. On the same figure one may see a follicle from which the colloid had been accidentally removed during sectioning: no reaction is observed there. In Pl. 1, fig. 5, three detached clumps of colloid may be seen in the upper left-hand corner, and a fragment of connective tissue in the lower left corner. A marked impression was produced on the autograph by the colloid, but none by the connective tissue. In order to make sure that the action of fixatives or reagents did not interfere with the results, the same examinations were performed with the same results in slides treated by the freezing drying method (Pl. 2, figs. 11, 12). In order to see if during the paraffin routine method some radio-iodine was extracted from the sections by the reagents, a measurement of the radio-iodine present in the latter was made with a Geiger counter, in the case of a few of the rabbits. Less than 20 % of the estimated iodine content of the thyroid fragments was found to be extracted by the fixative fluid and other reagents. It may therefore be concluded that most of the radioactive iodine is fixed in the colloid in a form which is not extractable by water, alcohol or xylol.

Further histological analysis of the data shows that all the follicles do not accumulate an equivalent amount of iodine. The denser the colloid, the greater is the amount of radioactive element fixed in it (Pl. 2, figs. 7, 8 and 9, 10). Since the denser colloid

usually stains with acidophilic dyes, there is a contrast between the acidophilic colloid accumulating large quantities of iodine and the basophilic colloid which contains smaller amounts of it. It seems that the presence of a basophilic rim around the eosinophilic material (rat), or its absence (rabbit), does not influence the iodine-fixing ability of the acidophilic colloid.

The variations in the time between the injection of radio-iodine and the autopsy of the animals did not modify the pattern of distribution of radio-iodine. However, the intensity of the reaction, as far as it can be estimated by this method, appeared smaller in the dogs examined half an hour after injection, thus indicating that less iodine was fixed by the thyroid in 30 min. than in 8 or 48 hr. when the radioactive element is given without carrier. In the rats given 5000 μg. of iodine per kg. body weight, for which only routine prepared sections were available, the radio-iodine was distributed in the same manner as in the other cases.

DISCUSSION

The presence of a greater amount of iodine in the colloid than in the cells was first established by indirect evidence, as it was noticed that the iodine content of the thyroids varied directly with the amount of colloid found in them (Oswald, 1902; Marine & Williams, 1908; Kocher, 1912). Later, Tatum (1920) separated cells and colloid by floating frozen sections of the thyroid in Ringer's solution, and centrifuging out the cellular material; the colloid remained in solution. Using this method, most of the iodine was found in solution along with the colloid material.

When minute physiological doses of iodine are administered, the results of this work show that iodine upon entering the thyroid gland is quickly stored in the colloid. In addition, most of this iodine is not washed off during routine histological procedures, and must therefore be combined in a rather insoluble form. This is in agreement with results obtained by chemical analysis, showing that the iodine fixed by the thyroid is transformed into the amino-acid diiodotyrosine, a component of the typical thyroid protein, thyroglobulin (Mann *et al.* 1942). Since the chemical results also suggested that diiodotyrosine is formed at the level of the outside membrane of the thyroid cell (Mann *et al.* 1942), it appears that the newly formed diiodotyrosine must be rapidly carried from the outside membrane to the colloid, where the autographs reveal its presence soon after injection.

When large doses of iodine comparable to those used in medical therapeutics were administered, the fate of this iodine in the gland was examined by Tatum's method. Van Dyke (1920–1, 1922) reported that a single injection of 50 mg. of iodide to the dog increased the iodine content of the colloid (confirmed by Nishikawa, 1930). With the help of radio-iodine, it was shown by the same method that, 30 min. after injection of a dose of radio-iodine of 5 mg. per kg. body weight, most of the radio-iodine was found in the colloid fraction (Leblond, 1942). However, since iodine injected in large amounts is first stored in the gland in a diffusible form (iodide), it may diffuse from the cells during sectioning and centrifuging. The localization of the iodine fixed as iodide by the thyroid glands after injection of a large amount of it is, therefore, not definitely established. In this work, rats given 5 mg. of iodine per kg. body weight were killed 24 hr. later. Their thyroids treated by routine histological technique produced autographs similar to those of animals receiving a small dose of iodine. Therefore, the iodine at that time was also fixed in an insoluble form; while, as stated above, half an hour

after injection of the same large dose, the iodine had not been transformed into diiodotyrosine, but was stored as iodide (Leblond & Süe, 1941). Even though the iodine may first be accumulated by the gland in inorganic form, a large fraction of this iodine is, within 24 hr., fixed in a stable form in the colloid.

CONCLUSIONS

1. After injection of a single small dose of iodine similar to the quantities of iodine available in physiological conditions, the autograph method shows that iodine is rapidly deposited in the colloid material of the thyroid gland.
2. A greater deposition of iodine occurs in the denser eosinophilic colloid.
3. The iodine is fixed in a form which is insoluble in the fluids used during routine histological technique, at least for the most part.
4. Even when a large dose of iodine is injected to the rat, a large amount of it is found in a stable form in the colloid 24 hr. after the injection.

We wish to acknowledge the help given by Dr W. Bale, Mr J. Bonner and Mr W. Mann, in setting up the freezing drying technique and preparing the autographs. For the radio-iodine we are indebted to the physicists of the University of Rochester and of the Massachusetts Institute of Technology, and especially to Dr Robley D. Evans.

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EXPLANATION OF PLATES 1 AND 2

Each microphotograph is accompanied by the corresponding autograph enlarged at the same magnification.

Figs. 1–10. Thyroids of 100 g. rats given 10 μg. of radio-iodine with autopsy 24 hr. later. The histological photographs were taken from paraffin sections stained by Masson's method.

PLATE 1

Figs. 1, 2. Showing that a blood vessel filled with blood cells does not react. Good reaction of colloid masses; no reaction from two tangentially cut follicles showing many thyroid cells. × 120.

Figs. 3, 4. Contrast between the thyroid tissue and the parathyroid gland visible in the upper part of the figures. × 30.

Figs. 5, 6. The autograph shows many spots corresponding to colloid accumulations. Three masses of colloid in the upper left corner reacted on the plate; they come probably from the empty follicles visible to the left part of the thyroid tissue. The empty follicles do not react on the plate. × 30.

PLATE 2

Figs. 7, 8. Darkly staining accumulations of colloid are visible, especially along the edges. They impress the plate markedly. × 30.

Figs. 9, 10. Compare the reaction of basophilic and eosinophilic colloid, along left lower edge. × 120.

Figs. 11, 12. Rabbit thyroid prepared by the freezing drying technique (H.-E. stain). Colloid accumulations act on the photographic plate.

Fig. 1

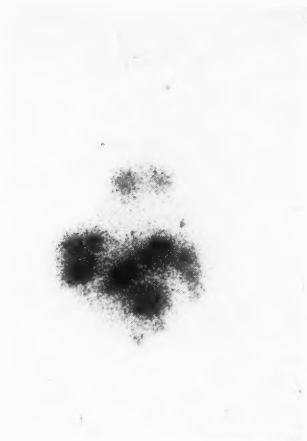


Fig. 2

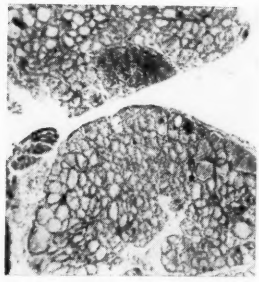


Fig. 3

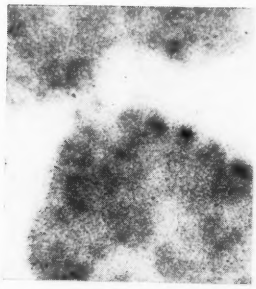


Fig. 4

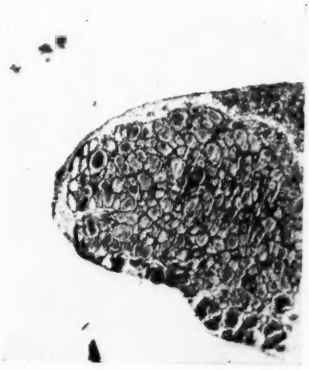


Fig. 5

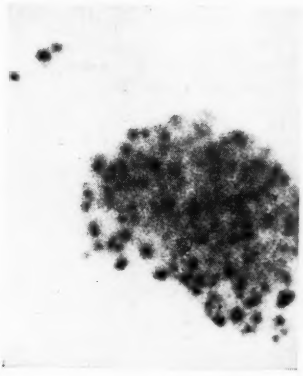


Fig. 6

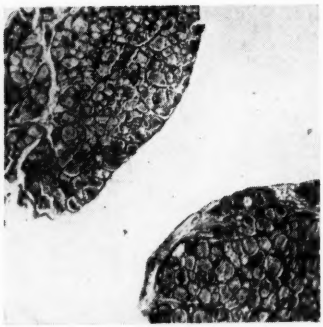


Fig. 7

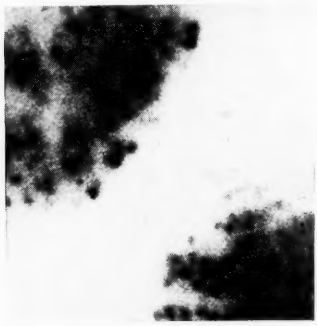


Fig. 8

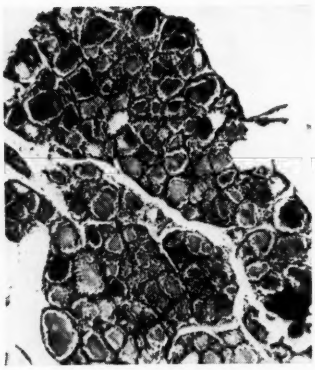


Fig. 9

Fig. 10

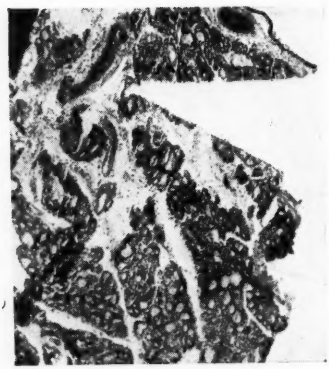


Fig. 11

Fig. 12

OBSERVATIONS ON THE STRUCTURE OF THE CONNECTIVE TISSUE SHEATHS OF CUTANEOUS NERVES

By P. GLEES, *Department of Anatomy, University of Oxford*

In a previous paper (Weddell & Glees, 1941) the process of the degeneration of the nerve fibres in the cutaneous plexus was described. However, the relation of cutaneous nerve fibres to their sheath elements during degeneration and regeneration was not dealt with, and relatively little is known about this even in the main nerve trunks. Spielmeyer (1922) showed that the general pattern of a nerve is not lost, even after a long period of degeneration. Holmes & Young (1942) have recently emphasized that each original neurilemmal tube ('Schwann tube') remains intact and able to lead regenerating fibres back towards the periphery. They investigated only the main nerve trunks. The present investigation is an attempt to discover whether a similar preservation of the sheath elements exists in a degenerated cutaneous nerve plexus. If they do so, then a pathway is provided by which regenerating fibres may be led back all the way to the original end-organ.

In the type of preparation commonly used to demonstrate nerve fibres in the skin the connective tissue elements and their relations to the nerve remain obscure. In the present investigation a study has been made of the structure and behaviour of the connective tissue elements and their relationship to nerve fibres in the normal, degenerating, and regenerating states, by means of stains specific for connective tissue, both alone and in combination with silver stains, and also by the use of polarized light.

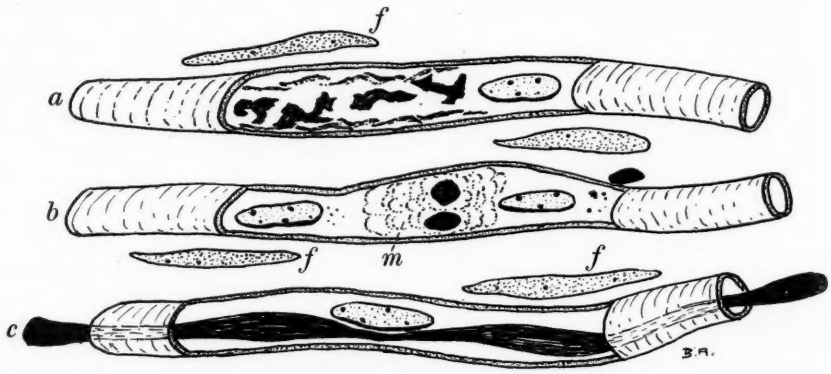
MATERIAL AND METHODS

This work was mainly done on skin taken from the rabbit, either from the ear or from the dorsum of the foot. Specimens of normal skin were taken from twenty rabbits. In a further series of fifteen rabbits the dorsal cutaneous nerve of the ear was crushed or sectioned and specimens of skin were taken 8, 14, 21, 40, 60 and 120 days after operation. In three rabbits a piece of skin was taken from the dorsum of the foot at intervals respectively of 4, 6 and 7 months after the peroneal nerve had been cut high up in the thigh. In addition to the work on the rabbits, preparations were made of a piece of human skin taken from a normal finger. All the specimens were fixed in 10% formalin or in Müller's fluid. The methods of silver and myelin staining used were those previously described by Weddell & Glees (1941). In order to study the connective tissue elements, Hortega's connective tissue stain and the Bielschowsky-Maresch impregnation method were employed. Silver sections were counterstained with van Gieson, light green, or a mixture of aniline blue and orange G. Polarized light was used in the study of the collagenous tissue.

THE NORMAL ANATOMY OF THE CONNECTIVE TISSUE SHEATHS OF NERVE BUNDLES IN THE SKIN

A fasciculus of a nerve bundle of medium size in the cutaneous plexus of a rabbit's ear contains 15–20 nerve fibres. These nerve fibres are enclosed by the perineurium, a connective tissue sheath formed mainly of collagenous fibres with scattered fibro-

blasts. The collagenous fibres of the perineurium are orientated in two main directions, (*a*) longitudinal fibres, and (*b*) oblique fibres arranged in a double spiral crossing one another at an angle of about 45 degrees, and interdigitating with the longitudinal fibres (Text-fig. 2). This whole assembly of fibres forms a compact network surrounding the nerve bundles. In addition to the perineural sheath each individual medullated nerve fibre has a connective tissue sheath of its own constructed of collagenous fibres running mainly in a longitudinal direction. With polarized light in a teazed preparation it is possible to observe that in a large cutaneous nerve trunk there are not only longitudinal connective tissue fibres surrounding each individual myelinated nerve fibre (see Text-fig. 1), but also very fine fibres running obliquely and crossing each other as in the perineurium. Polarized light also demonstrates clearly that the incisures of Schmidt-Lantermann in the myelin sheath are surrounded by localized



Text-fig. 1. Diagram showing three individual nerve fibres. In fibres (*a*) and (*b*) the axone and myelin sheath are undergoing degeneration. The degeneration takes place inside the endoneural tube, which in fibre (*b*) is dilated by a 'digestive chamber' formation. Fibre (*c*) shows the intra-endoneural position of a regenerating axone and illustrates the thinning out of the fibre as it passes a Schwann nucleus. *f*, fibroblast nucleus; *m*, macrophage.

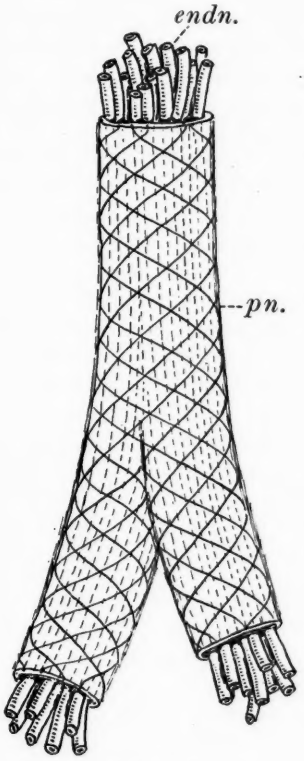
circular bundles of fibres (Text-fig. 3). However, by this method it is not possible to discriminate between collagenous fibres and argentophil fibres, since both show positive anisotropy (Schmidt, 1935). No argentophil fibres such as shown by Masson (1932) and Nageotte (1932) have been seen in the smallest cutaneous nerve bundles, though blood vessels running near these nerve bundles show the argentophil fibres quite clearly. I have not therefore been able to demonstrate any distinction between an outer and inner endoneurium, such as made by Plenk (1927) and Laidlaw (1929). Nor has it been possible to demonstrate a distinct inner membrane (Schwann membrane or neurilemma) such as has been described since the time of Schwann around the nerve fibres of main nerve trunks (see Holmes & Young, 1942).

The endoneural sheath of each myelinated nerve fibre encloses the Schwann cells, the myelin sheath, and the axis cylinder, and it is sometimes called the 'sheath of Key and Retzius', or, erroneously, the 'sheath of Henle'.

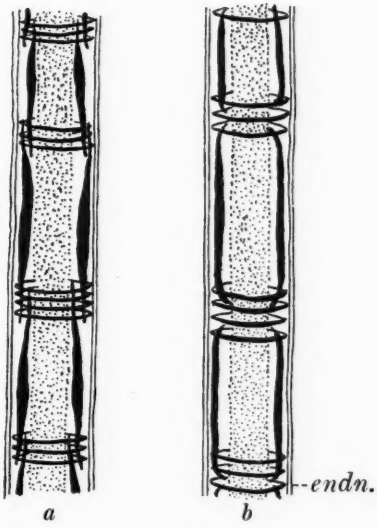
In the larger and medium-sized nerve bundles of the skin the perineurium encloses the nerve fibres within several layers of collagenous fibres. In smaller bundles con-

taining only two or three nerve fibres it may have only one or two collagenous layers. However, even in the smallest bundles the arrangement of longitudinal and oblique fibres in the perineurium described above is maintained.

The individual fibres of the endoneural sheaths are relatively coarse, and their diameter in the smallest bundle is the same as that in the large bundles or even in a small nerve trunk. In the smallest bundles, containing from one to five fibres, the



Text-fig. 2



Text-fig. 3

Text-fig. 2. Diagram showing the double spiral arrangement of collagenous fibres in the perineurium (*pn*). The individual endoneural tubes, surrounded by the perineurium, are also indicated (*endn*).

Text-fig. 3. Diagrams of fresh normal medullated nerve fibres as seen under polarized light. In (*a*) the incisures of Schmidt-Lantermann are seen, surrounded by circular bundles of connective tissue fibres. In (*b*) the fibre has been stretched and the cylindro-conical segments between the Schmidt-Lantermann clefts are seen to be extended. In both diagrams the axone is represented by stippling.

endoneurium of individual myelinated nerve fibres decreases slightly in thickness, although even single fibres running to a hair follicle or to the epidermis still have an endoneural sheath which can be distinguished from the thin perineurium right up to the end-formation. This description of the endoneurium and perineurium holds good not only for the rabbit's skin but also for human skin.

THE BEHAVIOUR OF PERINEURIUM AND ENDONEURIUM DURING DEGENERATION

The break-up of the axis cylinder and the myelin sheath and the early changes of degeneration in cutaneous nerve fibres have been dealt with in a previous paper (Weddell & Glees, 1941), and we can therefore proceed to describe a more advanced phase of degeneration corresponding to Text-fig. 1 *a*, *b*. In the latter, myelin and axone are in various phases of degeneration. In the large 'digestive chambers' of Cajal remnants of the axones are still visible and macrophages are to be seen removing the debris. The endoneural sheath, showing conspicuous collagenous fibres, is dilated wherever digestive chambers have been formed. In those parts of the endoneural tubes where no digestive chambers have developed, the endoneural tube is reduced in diameter and appears to have collapsed. When the endoneurium has become completely free from the last remaining fragments of axone and myelin, hypertrophy of the Schwann protoplasm sets in. It seems probable that the endoneural tube of the peripheral branches is filled out by the enlargement of individual Schwann cells rather than by their active proliferation.

Four or five weeks after section, every endoneural tube or 'Schwann tube' (Holmes & Young, 1942) of the peripheral cutaneous bundle is filled with the so-called Schwann bands. The formation of these Schwann bands leads to the result that the cutaneous nerve bundles, emptied of their former contents (the axis cylinder and myelin sheath), almost regain their original diameter. This applies not only to the individual endoneural tubes but also to the whole contents of the perineurium. It is of the greatest interest in the consideration of the whole question of regeneration to ascertain how long the framework of the cutaneous nerve plexus can persist without being re-innervated. In pieces of skin which had been kept denervated for 4, 6 and 7 months, perineurium, endoneurium and Schwann cells could be easily followed to their terminal organs and the general amount of shrinkage was slight. The endoneural tubes of skin kept denervated for 7 months were of uniform thickness and stained more darkly than those kept denervated for shorter periods. It thus appears that by this time the wall of the tube has become more compact. The diameter of an endoneural tube of the cutaneous plexus which has been long denervated is about two-thirds that of a normal tube. According to Holmes & Young (1942) the diameter of an endoneural tube of a peripheral stump may even decrease to one-half. The perineurium, however, seems unchanged both in the number of its layers and in its thickness.

Long-term degenerated peripheral stumps and their histological structure have been described by Spielmeyer (1922) and quite recently by Holmes & Young (1942). The former describes a peripheral stump which had not been re-innervated 2 years after section, and in which the endoneural connective tissue was well preserved. He even believed the collagenous tissue to have increased to a certain extent. The Schwann cells were found still to be intact. Spielmeyer also refers briefly to a few cases where distal nerve trunks had maintained their general architecture, even as long as 3 or 4 years after section. The general structure was well recognizable in spite of a certain amount of shrinkage of the distal stump. These observations have been confirmed by Holmes & Young (1942).

THE RELATION OF THE ENDONEURIUM TO OUTGROWING NERVE FIBRES

If a nerve is crushed experimentally, the newly grown fibres will reach the periphery earlier than if the nerve has been cut and sutured (Gutmann, Guttmann, Medawar & Young, 1942). In the early stage of regeneration the pathways are still partly blocked by debris and by macrophages which are occupied in clearing it. The newly formed fibres are fine thin threads of small diameter and run in close relation to the Schwann cells (Spielmeyer, 1922; Boeke, 1935). The new fibres in the cutaneous bundles of the skin are thinner at the points where they pass digestive chambers. It would be difficult to explain this local thinning if the course was extra-endoneural, for the thinning of fibres is evidently a compression effect due to the almost complete blockage of the endoneural tubes. It may be inferred, therefore, that the regenerating fibres certainly have an intra-endoneural course. A similar thinning out can be observed where the new nerve fibre has to pass the Schwann nucleus lying inside the endoneural tube (Text-fig. 1 *c*). Indeed, an individual fibre can frequently be traced from the lesion right the way to the terminal destination, travelling within the same endoneural tube. Where the tube branches, the fibre it contains branches as well. It may thus be concluded that the regenerated fibres tend to be guided to the periphery by the endoneurium and, once having entered a tube, are forced to pursue the course followed by the tube in all its ramifications.

DISCUSSION

In attempting to analyse the structural significance of the connective tissue elements from a mechanical point of view, it may be pointed out that the spiral arrangement of fibres in the perineurium of the cutaneous nerve bundles is well suited to allow for extension. This arrangement can also be detected in the endoneurium of bigger bundles, in which the fibres show the same disposition to cross obliquely. However, if this arrangement of the perineurium and endoneurium is purely an adaptation allowing for extension, it must be shown that the myelin sheath and the axis cylinder can also be extended. A possible arrangement to permit extension of the myelin sheath lies in the incisures of Schmidt-Lantermann. These structures, by dividing the internodal segments into a number of cylindro-conical parts, might permit extension of the myelin sheath in response to bending or pulling by sliding in and out of one another like the tube of a telescope. Any weakness of construction at this particular point is obviated by a special system of circular fibres surrounding the incisures. Moreover, the incisures of Schmidt-Lantermann are found only in the myelin sheath of the peripheral nervous system where stretching is likely to take place and they are absent in the brain and spinal cord which are relatively more fixed. If the incisures exist solely for passing nourishment along to the axis cylinder (as some authorities have suggested), it might be expected that they would be present in the central nervous system as well. In order to verify the suggestion that the incisures allow extension, a piece of peripheral nerve was stretched under direct observation of the polarization microscope (Pl. 1, figs. 4, 5). If the nerve fibres are stretched, the myelin segments between the incisures become separated from each other and when relaxed they slip back into the original position. It may be noted that the extension of the endoneurium

and perineurium, which was also observed to take place, is accompanied by a decrease in the anisotropy of the constituent collagenous fibres.

It has been observed that in denervated skin the role of the connective tissue sheaths and Schwann cells is to preserve the pattern of ramification should re-innervation occur. As already mentioned above, Spielmeyer and Holmes & Young (in more detailed observations) found that after denervation the peripheral stumps still persist and the assumption follows that the perineurium and endoneurium of the cutaneous plexus can also survive for a similar length of time. The most striking example of denervated skin that the writer was able to examine was the skin of a rabbit's foot which had been denervated 7 months previously. It was found possible to distinguish the entire connective tissue elements and the Schwann cells of the cutaneous plexus, which were in a good state of preservation. In this connexion attention should be drawn to a paper by Kadanoff (1925). Kadanoff transplanted the skin of the rabbit's sole into the region of the snout, which has a much richer and more complicated nerve supply and a different arrangement of the nerve plexus to that found in the skin of the sole. He was surprised to find that the regenerated fibres of the snout in the transplanted skin of the sole assumed the more simple plexus formation of the donor region. On the other hand, in the light of our observations it is clear that the newly ingrown fibres of the fifth nerve were forced to follow the preserved pattern of the sole type. Kadanoff's technique, however, demonstrated only the nerve fibres without their sheath elements, and he was thus unable to observe the persisting framework of the plexus. It is most important to realize that the mode of branching is fixed by the connective tissue sheaths forcing definite pathways upon the regenerating nerves. The individual character of a nerve fibre can only be expressed by those terminal arborizations which are free from sheath elements. In this particular case Kadanoff found that the end formations in the transplanted specimen were actually more complicated than is usual in the normal skin of the sole.

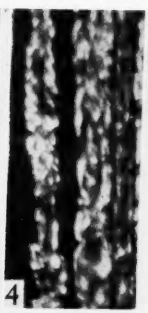
The new fibres grow down inside the endoneural tubes to their termination. This can be clearly demonstrated in the larger bundles, but even where single fibres are growing towards hair follicles their intra-endoneural course is observable. It is evident from our preparations that the whole peripheral nervous system (with the exception of some terminal ramifications) is enclosed in a tubular formation which completely separates the nerve fibres from the neighbouring tissues. In this connexion an observation by Lashkov (1938) may be noted, namely that the perineurium can be followed in continuity with the capsular connective tissue apparatus of sensory corpuscles in the human skin. This conservation of pattern greatly facilitates the progress of the new ingrowing fibres which could otherwise hardly reach their goal at the end-organs if they had to struggle in the complicated meshwork of collagenous fibres inside the skin.

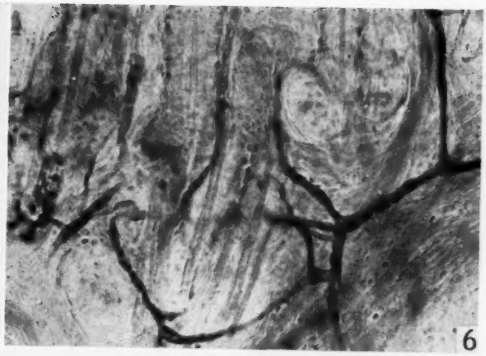
SUMMARY

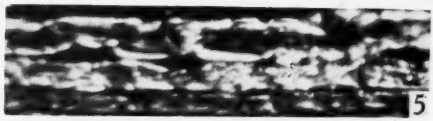
1. The perineurium and the endoneurium are essential parts of the cutaneous plexus and can be followed up to the terminal arborizations of the nerve fibres.

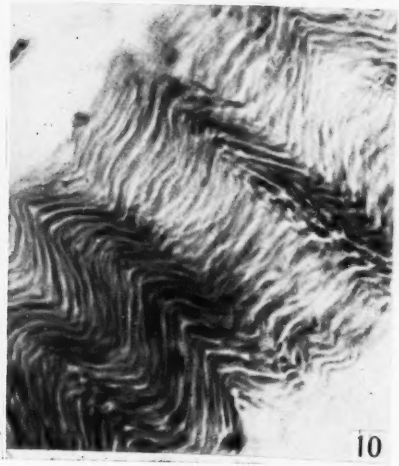
2. The perineurium consists of collagenous fibres arranged longitudinally and obliquely. The latter decussate to form a double spiral. Each nerve fibre has its individual endoneural sheath.

3. The endoneurium consists of longitudinal and decussating fibres in the larger









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nerve bundles and of longitudinal fibres only in the smaller bundles. The endoneurium ensheathes the nerve fibre right up to the end-organ.

4. The incisures of Schmidt-Lantermann in the myelin sheath appear to be a mechanism for permitting extension and are surrounded by a special circular bundle of fibres.

5. Degeneration of the myelin sheath and axis cylinder, occurring within the endoneural tubes, seems to lead partly to the dilatation of the latter and partly to their collapse. After the hypertrophy of the Schwann cells, the tubes return approximately to their previous dimensions.

6. Perineurium, endoneurium and hypertrophic Schwann cells together preserve the old pattern of innervation for the new ingrowing fibres. These new fibres grow down inside the old endoneural tubes and are thus forced to repeat the former mode of ramification to a given area of skin.

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EXPLANATION OF PLATE 1

Figs. 1, 2, 3. Normal medullated nerve fibres seen under polarized light, showing the incisures of Schmidt-Lantermann. The endoneural sheath is seen in fig. 1. ×700.

Figs. 4, 5. Normal medullated nerve fibres subjected to tension by pulling on both ends, and seen under polarized light. Note that the cylindro-conical segments of Schmidt-Lantermann have become extended. ×700.

Fig. 6. Section of the skin of a rabbit's ear which has been kept denervated for 2 months. The whole pattern of ramification is preserved right up to the hair follicles. Modified Bielschowsky stain. ×80.

Fig. 7. Section of normal skin of a rabbit's ear, showing the connective tissue sheaths of a cutaneous nerve bundle. Hortega's stain for connective tissue. *pn*, perineurium; the endoneurium is shown above. ×450.

Fig. 8. Transverse section of a human cutaneous nerve containing about 20 axones. Note the ensheathing perineurium and, faintly outlined, the endoneural sheaths. A modified Bielschowsky method. ×400.

Fig. 9. A branching cutaneous nerve bundle from a rabbit's ear which had been kept denervated for 2 months. The endoneural tubes are filled with the 'Schwann bands'. A modified Bielschowsky method. ×820.

Fig. 10. The endoneural tubes, filled with the 'Schwann bands', of a cutaneous nerve bundle from the skin of the dorsum of a rabbit's foot which had been kept denervated for 6 months. A modified Bielschowsky method. ×160.

THE STAINING REACTIONS OF NORMAL SYNOVIAL MEMBRANE WITH SPECIAL REFERENCE TO THE ORIGIN OF SYNOVIAL MUCIN

By D. V. DAVIES, *Anatomy School, University of Cambridge*

Several theories have been advanced to explain the origin of synovial fluid, and these have been summarized in a recent review by Bauer, Ropes & Waine (1940). No theory, however, is complete that does not explain the seat of origin of the synovial mucin to which the fluid owes its most outstanding property—its viscosity. A study of the literature concerning this substance makes it clear that there is no unanimity of opinion concerning the staining reactions of the synovial membrane. Thus Cherry & Ghormley (1938), using the mucicarmine method described by Mayer (1896), claim to be able to demonstrate mucin concentrated in or about the nuclei of the synovial cells, and these workers believe that the mucin gains entrance into the joint by degeneration of the synovial cells. A similar view is held by Policard (1936). Mayeda (1919–20) and Kling (1938), however, have failed to stain any secretory cells by the mucicarmine technique. Fisher (1923) claims that the mucin-secreting cells can be demonstrated with carbol thionin, and states that 'every stage of transition between well-marked granules and definite droplets may be seen', and, further, that in some cases 'cells distended with mucin and the nucleus compressed' could be observed. This description suggests the presence of goblet cells in the synovial membrane, a view previously put forward by Soubbotine (1880). Kling, on the other hand, is unable to stain the mucin-secreting cells with carbol thionin but claims that these cells can be stained metachromatically with toluidine blue with the aid of a special mountant consisting of gum arabic, saccharose and water made alkaline with ammonia. This avoids passage of the sections through the alcohols which are said to destroy the metachromatic granules.

In a study of synovial cells growing on a plasma coagulum Vaubel (1933) found that they showed a marked tendency to liquefy the substrate and that they produced a mucin-like substance. This substance was precipitated by acetic acid and was insoluble in excess of acid but soluble in alkalis. The quantity of precipitate obtained did not allow of further chemical examination for its identification. Vaubel did not succeed in staining the cultured cells with mucicarmine and mucihaematin, and only occasionally were metachromatic granules found in the cells on staining with toluidine blue.

Our knowledge of the distribution, chemical properties, and constitution of the mucopolysaccharides is still very meagre. Recent work by Duran-Reynals (1929), Chain & Duthie (1939), McClean (1941), Madinavietia & Quibell (1940), Meyer (1938) and others on the action of diffusing factors and their mucolytic activity has added new interest to this group of proteins. Meyer & Palmer (1936) isolated the viscous component of synovial fluid, vitreous humour and Wharton's jelly, and named this sulphur-free compound hyaluronic acid. Duran-Reynals, while studying the effect of tissue extracts on viruses, found that the extent and rate of spread of particulate matter and bacteria in the tissues were greatly increased by the addition of watery extract of the testicle to the injected matter. The active substance in these extracts was termed the 'spreading factor'. Substances with a similar spreading action have

been identified since in bacteria, snake venoms and malignant tumours. Chain & Duthie identified in these testicular extracts an enzyme, hyaluronidase, capable of hydrolysing the sulphur-free hyaluronic acid. It is still uncertain whether the spreading factor of Duran-Reynals and the enzyme, hyaluronidase, are identical. Recent workers incline to the view that they are of different origin. The discovery of the mucinase, hyaluronidase, has, however, placed in our hands a new weapon for the study of the sulphur-free mucins.

MATERIAL AND METHODS

Normal synovial membrane has been studied. This has been obtained from a wide variety of sources, as the writings of Kling (1938) and King (1935) suggest that marked differences may be found in the various animals. Joint membranes from the following sources have been studied: man, *Macaca mulatta*, rabbit, rat, mouse, cat, and cattle. Synovial membrane has been drawn from the knee, ankle or hock, shoulder and radiocarpal joints, care being taken to examine several different areas in each of these joints.

Various fixatives have been tried. These have included 5 and 10 % formalin, formol acetic, Zenker's fluid (with and without acetic acid), Carnoy's fluid, saturated mercuric chloride (with and without 5 % acetic), Susa's fixative, and 3 % basic lead acetate. Holmgren & Wilander (1937) found the last named particularly suitable for the demonstration of metachromatic granules in mast cells.

The stains have included toluidine blue, methylene blue, polychrome methylene blue, cresyl violet, thionin, carbol thionin, safranin, mucicarmine (Mayer, 1896; Southgate, 1927, and Rawitz, 1899) and mucihaematin (Mayer). In the case of the metachromatic dyes, the modifications suggested by Hoyer (1890), Lillie (1929), Bruno (1905), Hari (1901), and, in the case of safranin, Kultschitzky (1897), have been used in addition to the standard methods. Where available, the dyes used were produced by the firms of Gurr, Flatters and Garnett, and Grubler. Several sections stained with haematoxylin and eosin have also been prepared.

Frozen and paraffin sections were examined. These were cut at 5–10 μ and, in cases where the metachromatic stains were used, were examined in water before mounting in Canada balsam, Farrant's mixture, Gurr's neutral mounting medium, or in a medium similar to that recommended by Kling. The brand of gum arabic suggested by Kling could not be obtained. In all some hundreds of sections were prepared with careful control by parallel treatment of sections of large gut and human umbilical cord.

In some cases sections stained with toluidine blue were prepared after incubating the sectioned tissue with a concentrated solution of hyaluronidase (testicular extract) at *p*H 4·6 and 37° C. Others were incubated overnight with a large volume of half-saturated lime solution in normal sodium chloride according to the method used by Bensley (1934), but omitting the continuous drip of lime water. On the control gut sections it was found that by this method the mucin could be extracted without at the same time removing the cellular elements and connective tissue fibres.

RESULTS

As it is impossible to give in detail the results for each method and modification used, the description of sections will be confined to those stained with Southgate's mucicarmine, Mayer's mucihaematin and with polychrome methylene blue after fixation with Susa's fixative, embedding in paraffin and mounting in Canada balsam in the usual manner. Using gut as a control, these were found to be the most satisfactory

methods for the demonstration of mucin in the goblet cells. In addition, the results of treatment with alkali and with hyaluronidase will be described.

(1) *Synovial membrane stained with Southgate's mucicarmine*

The most striking feature in these sections was the greater depth of staining of the synovial cells immediately adjacent to the joint cavity; this was exaggerated in the more cellular areas of the synovial membrane (Pl. 1, fig. 1). A similar enhanced affinity for this stain was also noted in the middle and outer coats of the arterioles and larger blood vessels of the synovial membrane. The cytoplasm of the cells in these two areas was stained a uniform dark red colour, but the tint of the staining was never that bright red seen in the goblet cells of the gut. The nuclei of the synovial cells were not stained with mucicarmine but took on the colour of the basic dye, in this case haematoxylin. No cell resembling a goblet cell was seen in the synovial membrane. The red staining of the nuclei described by Cherry & Ghormley (1938) was not seen, even in the pathological specimens examined as, for example, after the injection of a 5 % calcium chloride solution into the knee-joint of a rabbit which was killed 12 hr. later. Possibly the failure of the nuclei to stain red was due to the use of a different brand of mucicarmine. In the normal membrane the ground substance and intercellular fibres were stained faintly pink. No vacuoles were seen in the cytoplasm of the synovial cells.

No change was detected in the staining reactions of the synovial membrane with mucicarmine after incubating the sections with a solution of hyaluronidase at *p*H 4·6 and 37° C. overnight or after treating the sections with a half-saturated solution of lime water in normal saline. Control sections of gut treated in a similar manner with half-saturated lime water showed that the mucin in the goblet cells had been removed and a basophilic cytoplasmic network left behind in place of the mucin droplet.

(2) *Synovial membrane stained with mucihaematin*

Similar results to the above were obtained with Mayer's mucihaematin. The cytoplasm of the lining synovial cells and underlying connective tissue cells was stained a uniform greyish blue colour with this dye. This was unaffected by treatment with weak alkali or by incubation with a solution of hyaluronidase under the same physical conditions as with mucicarmine.

(3) *Synovial membrane stained with metachromatic dyes*

Using gut and umbilical cord sections as controls, the best results with polychrome methylene blue, and indeed with all the metachromatic dyes, were obtained when:

(i) The material was fixed with a mercuric salt solution. Susa's fixative and saturated mercuric chloride solution alone, or with 5 % acetic acid added, were found equally suitable. Basic lead acetate, as suggested by Holmgren & Wilander (1937) for staining of mast cells, not only gave poorer fixation (even of the mast cells) than the mercuric fixatives, but also made the sections brittle and difficult to cut as well as leaving a dirty precipitate scattered haphazardly through them.

(ii) The dyes were used in high dilution, that of the order of 1/64,000 being most satisfactory. The dilution was made immediately before use from a 1 % stock solution. Satisfactory staining developed in 20–30 min. Of the metachromatic dyes used, toluidine blue and polychrome methylene blue gave the best results.

The sections were examined in water before mounting. In most cases they were then passed rapidly through the alcohols and mounted in Canada balsam. Passage through the various grades of alcohol was not harmful, provided it was not unduly prolonged. A brand of gum arabic was found which caused the sections to develop metachromasy as described by Kling (1938), but no advantage could be found in this method. The mountant was discarded as empirical and of doubtful significance.

No advantages were found in the use of the modifications suggested by Lillie, Hóyer, Bruno, Hari or Kultschitzky. Iodization of sections fixed with mercuric chloride did not have any deleterious effect on the development of metachromatic staining.

Briefly, the best results were obtained as follows. Fresh material was fixed with a mercuric solution, iodized, cut in paraffin at 5–10 μ, dewaxed and stained in a very dilute solution of the metachromatic dye, passed rapidly through the alcohols and mounted in Canada balsam.

With these dyes the staining reactions of the lining synovial cells and the underlying connective tissue cells were similar. The nuclear chromatin stained a deep blue while the cytoplasm of the cell remained unstained or was stained a very faint blue. The intercellular fibres remained unstained while the endothelium of the blood vessels and capillaries stained a deep blue. In no case was any metachromatic staining of the lining cells obtained, but scattered through the underlying connective tissue were large polyhedral cells which showed metachromasy. This appeared either as a coarse metachromatic granulation or as a diffuse uniform metachromasy of the cytoplasm. The cells were relatively few in number and were arranged mainly along the blood vessels (Pl. 1, fig. 2). No cells were found discharging their contents at the surface of the synovial membrane. Furthermore, the metachromatically staining cells preserved their staining reactions after incubating the sections with a hyaluronidase solution at *p*H 4·6 and 37° C. for 12 hr. and after treatment with a half-saturated solution of lime water. No cells were found in the synovial membrane bearing any resemblance to the goblet cells of the gut. Control sections of gut stained with the metachromatic dyes lost their distinctive reaction after treatment with half-saturated lime water overnight. In the umbilical cord sections streaks of metachromatic staining were found in the ground substance; these were lost after incubation with half-saturated lime water. The polyhedral cells staining metachromatically in the synovial membrane were not found in sections of umbilical cord.

With the exception of the rabbit, no species differences in staining reactions were found. In the knee joint of the rabbit a diffuse area of metachromatic staining was found in the anterior wall of the suprapatellar pouch starting a short distance above the patella. This area was distinct from the cartilage of the patella which also stained metachromatically, and in it both the cytoplasm of the cells and the intercellular substance stained metachromatically (Pl. 1, fig. 3). This did not occur in any of the other species examined.

(4) *Synovial membrane stained by other methods*

(*a*) ***Golgi body.*** King (1935) states that Golgi bodies can be demonstrated in human synovial cells by de Fano's (1920) method, but the author could not find them in the human synovial membrane nor in any of the other material used. Control sections of gut showed the Golgi apparatus clearly.

(*b*) ***Mitochondria.*** The method of Bensley and Cowdry (1918) was used. Mitochondria

were seen in both the lining cells and the underlying connective tissue cells of the synovial membrane (Pl. 1, fig. 4). They were few in number, perhaps a little more numerous in the cells of the villi. They were scattered irregularly through the cytoplasm and did not exhibit any polarity. It may be of interest to note that no mitochondria were found in the cells of the umbilical cord.

DISCUSSION

Stormont (1928) lists the properties of mucus-secreting cells. Briefly these are:

(1) Presence of minute droplets of low refractive index in the cells.

(2) Metachromatic staining with thionin, toluidine blue, safranin, cresyl violet and other dyes.

(3) Red staining with Mayer's mucicarmine, blue staining with Mayer's mucihaematin.

(4) Staining with mucihaematin as indicator and using weak alkali to dissolve out any mucin.

(5) Paucity of basophilic material in the cell. Traces of this are shown by McCallum's microchemical reaction for iron.

(6) Absence of intracellular secretion canaliculi.

It is very questionable whether any one of these criteria is specific, and it must be admitted that there is still no microchemical test for the mucins. It is difficult to state definitely what chemical or physical tests should be considered characteristic of the mucoproteins; it is doubtful whether any one test alone is specific. Three characteristics of these substances might suffice for diagnostic purposes:

(*a*) They are proteins associated with a prosthetic carbohydrate radicle. This latter can be detected, after hydrolysis, as a reducing sugar.

(*b*) They are precipitated by acetic acid and are insoluble in excess acetic acid but soluble in alkali.

(*c*) They are all characterized by the formation of viscous solutions.

It is doubtful if one is justified in calling a substance mucin unless all three tests are positive, yet not one of these tests has its counterpart in any histological technique for the demonstration of mucin. An attempt was made to translate the first characteristic into a microchemical reaction by testing for the uronic acids after incubation with hyaluronidase for the purpose of hydrolysis, but this was unsuccessful. Several factors might account for this:

(*a*) The mucin may occur only in the joint and not in the membrane. This aspect will be discussed later.

(*b*) The amount of mucin may be so small as to produce insufficient reducing sugar for detection by any histological means.

(*c*) The liberated carbohydrate may be extracted from the tissues by the substrate as soon as it is formed.

There is no reason to suppose that a hyaluronidase solution will not act on the mucin in thin sections, as a similar test for glycogen, using saliva as a source of ptyalin, is quite satisfactory and perhaps at present the only safe histological test for glycogen using a carmine as an indicator. Until a method along these lines is devised, or until there is more detailed knowledge of the chemistry of the mucins, the histological tests for these compounds in tissues are bound to be unsatisfactory. Hyaluronidase is found to cause no change in the staining reaction of synovial membrane, suggesting

either that no mucin is present or that it does not stain by these methods. Fixation may affect the validity of such an investigation but the results of treatment of the control sections with alkalies suggests that this is not the case.

As regards the histological tests for mucin in the synovial membrane, investigations along the lines indicated in the second, third and fourth criteria of Stormont have yielded consistently negative results. Fisher (1923) claims to have demonstrated mucin with carbol thionin, Kling with toluidine blue but not carbol thionin, and Vaubel (1933) describes occasional cells showing metachromatic material in tissue cultures of synovial membrane.

The polyhedral cells which stain metachromatically in the synovial membrane are not mucus secreting cells, but must be regarded as mast cells since:

(*a*) they have the typical morphological appearance and staining reactions of mast cells,

(*b*) their distribution along the blood vessels is typical of mast cells,

(*c*) none of these cells has been seen to pour any secretion into the joint cavity nor indeed have they actually been observed amongst the lining cells of that cavity, but always placed a little deeper,

(*d*) they are unaffected by lime water, dilute alkalies and, most important of all, the enzyme hyaluronidase,

(*e*) metachromatic staining is only obtained with esters of sulphuric acid of high molecular weight.

The mucins can be conveniently divided into two groups, (1) the sulphur-containing, and (2) the sulphur-free. According to Lison (1935) only the former gives the characteristic metachromasy with the dyes used here, while the mucin of the synovial fluid belongs to the latter group and should not therefore give this reaction. It is interesting to note that certain parts of the ground substance of the umbilical cord, as distinct from the cells, contain cells which show metachromasy. This reaction is lost after treatment with weak alkali. This is in agreement with the results of Meyer & Palmer (1936) who describe both sulphur-containing and sulphur-free mucopolysaccharides in the umbilical cord. Concerning the functions of mast cells in this situation, Jorpes (1939) states that they inhibit the clotting of blood in the joint cavity. It is not known on what experimental evidence this statement is based, but the writer's experience is that shed blood clots very readily in all joints—normal or pathological—and synovial fluid more often than not seems to hasten the clotting of blood in vitro. Failure of synovial fluid to clot can be explained by its deficiency in fibrinogen.

Cherry & Ghormley (1938), using the mucicarmine technique of Mayer, claim to be able to demonstrate mucin in the synovial cells. They state that the synovial mucin is a product of degeneration of these cells. This is not in agreement with the results of other workers. Little is known of the mechanism of staining with mucicarmine. Seki (1933) states that carmine is positively charged with aluminium ions and stains like a basic dye, i.e. it stains the negatively charged elements in the tissue. It is not known whether aluminium in this stain has any laking effect. Mucin, whether sulphur-containing or not, should stain with mucicarmine if the above is the correct explanation of the mechanism of staining. The lining cells of the synovial membrane certainly stain a uniform dark red colour with mucicarmine, but despite this it is impossible to agree with Cherry and Ghormley's deductions for two reasons:

(*a*) The degeneration of synovial cells described in their pathological material was

not seen in normal synovial membrane. At no time in normal synovial membrane did the nuclei stain red with mucicarmine.

(*b*) The staining reactions of normal tissue were not altered by treating the sections with hyaluronidase or with dilute alkali. Thus the substance in the cytoplasm which stains red with mucicarmine cannot be chemical mucin.

Concerning the Golgi body, the results of King have not been confirmed. Furthermore, the main objection to this type of evidence is that the functions of the Golgi body are at present imperfectly understood and too debatable to be of value in the settlement of this question. King believes that a well-marked Golgi body is found only in the human synovial membrane and, like Kling, suggests that species differences occur in the mode of action of the synovial cells. It is doubtful whether such differences exist. Apart from the case of the rabbit already described, the writer could not find species differences in the morphological characters or the staining reactions of the synovial membrane in the types examined. No species or organ differences were found by Meyer & Chaffee (1941) in the hyaluronic acid prepared from the vitreous humour of pig, cow, a human tumour and bovine synovial fluid.

The objections that apply to the Golgi body are equally true as regards mitochondria. The mitochrondria of synovial cells differed little from that of the underlying connective tissue cells while at no time could any suggestion of polarity be seen. Even more interesting perhaps is the failure to demonstrate mitochondria in the cells of the umbilical cord with all its rich supply of mucopolysaccharides of two different types.

There seems, therefore, little to justify the claims made by Fisher, Kling and others that there are secreting cells in the synovial membrane, and certainly there is no evidence for the presence of special secretory areas in the synovial membrane, as suggested by Kling.

The following points in Vaubel's work certainly require clarification before his views can be accepted:

(*a*) Is the acetic acid precipitate test applied for mucin adequate? We know too little of the products of fibrinolysis in tissue cultures to be certain that no other substance is formed which reacts with acetic acid in a similar manner to the mucins.

(*b*) What is the explanation of the metachromasy occasionally obtained with toluidine blue?

(*c*) What is the factor in splenic extracts which leads to increased mucin production in the tissue culture?

Aqueous splenic extracts have been found to be a more potent source of a hyaluronidase-like substance than even the iris and ciliary body. However, in Vaubel's work the production of mucin in vitro has been obtained with greatest ease after the addition of splenic extracts to the cultures. Are we to assume that the splenic extracts used here were free of hyaluronidase or were the physical conditions (*p*H, etc.) such that the enzyme failed to act? McClean's work is interesting in this respect. He found that the inclusion of hyaluronidase in the culture medium for streptococci prevented the development of the capsules.

It appears that histological evidence concerning the origin of synovial mucin is based on very insecure grounds and is of little value in determining the source of the substance. Perhaps the greatest advance towards the solution of this problem will be obtained along the lines already indicated by Vaubel.

The biochemistry of the mucins is equally confusing. Little is known of the source and mode of production of the uronic acids in the body. The only identified uronic acid in the human is glycuronic acid. Quick (1926) states that glycuronic acid can be produced from glycogen and that if the supply of the latter is insufficient it can be formed more readily from protein or, more probably, from circulating amino-acids than from α- or β-glucose. It is interesting to note here that the administration of glucose seems to accelerate the excretion by the kidney of soluble glycuronates. The volatile oils are excreted from the human body as glycuronates. The rate of excretion is increased by the administration of glucose. Bywaters (1937) suggests that the concentration of synovial mucin varies with the glycolytic activity of the fringes. Against this view, it can be affirmed that no glycogen could be demonstrated in the synovial membrane of young cows. Other workers doubt if some animals can synthesize glycuronic acid and suggest that it is ingested preformed.

The mucin particles may be so large as to prevent their escape from the joint cavity, in which case their continued formation by the synovial membrane will be unnecessary. In this respect the deductions of Bauer, Ropes & Waine (1940) are interesting. They calculate that the osmotic pressure of mucin per gram is nine times as great as that of albumin. This would suggest a correspondingly small molecule for mucin, in which case its escape from the joint cavity should be comparatively easy. Molecules much larger than those of albumin can be removed with ease from the joint (Adkins & Davies, 1940). This suggests a continued formation of mucin in the joint to maintain the level of this substance. However, such deductions are not justifiable since several factors, such as solvation and *p*H, have to be taken into account. In fact it is probable that the mucin molecule is larger than is suggested by the above. The osmotic pressure of synovial mucin and fluid needs to be thoroughly investigated. The rate of mucin production may be so low that it is impossible to detect it with any ease histologically. This aspect of the problem is at present under investigation. As suggested by Vaubel (1933), the mucin in the joint is probably of the nature of an inert intercellular matrix and its formation might well slow down with age and perhaps finally cease altogether.

The inertness of the mucins chemically and biologically suggests that their primary function is skeletal and of the nature of a matrix. The distribution of mucin is also interesting. In the umbilical cord, the vitreous, and synovial fluid it is distributed chiefly in relatively avascular tissues, whose function is apparently purely mechanical. It is impossible to say whether its formation could be correlated with the avascular nature of the situations in which it is formed, but this certainly is suggestive.

In conclusion it can only be stated that our present knowledge of the synovial membrane is biologically as inadequate as our chemical knowledge of the mucins. The advance made by Chain & Duthie's discovery of hyaluronidase (1939) in the tissues may provide a more fertile approach to the problem.

SUMMARY

The current views concerning the origin of the synovial mucin are reviewed. The bearing of the recent discovery of an enzyme, hyaluronidase, capable of hydrolysing this mucin, on the problem of the origin of this substance is discussed.

The staining reaction of the synovial membrane with the mucicarmines and the metachromatic dyes both before and after treatment of the tissue with this enzyme

are described. The cytoplasm of the lining cells of the synovial membrane is stained a uniform red with mucicarmine, but this reaction is unaffected by incubation with hyaluronidase or alkalies. There is no confirmation that this staining reaction is due to mucin. The metachromatically stained cells in the synovial membrane are identified as mast cells. No cells containing either mucin or pre-secretion granules of this substance have been seen.

A course is suggested along which further work on this controversial problem of the origin of synovial mucin might follow.

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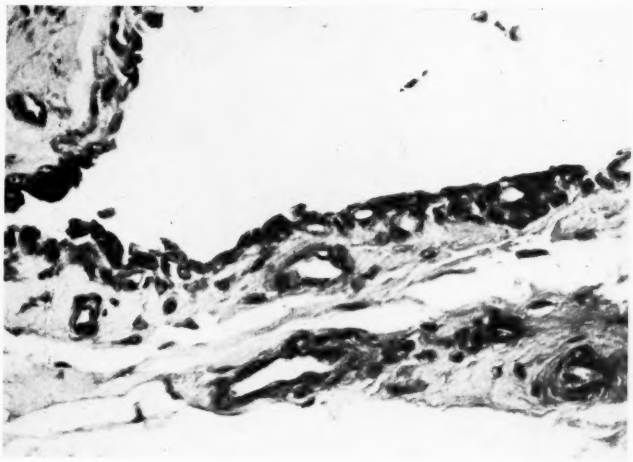

Fig. 1

Fig. 3

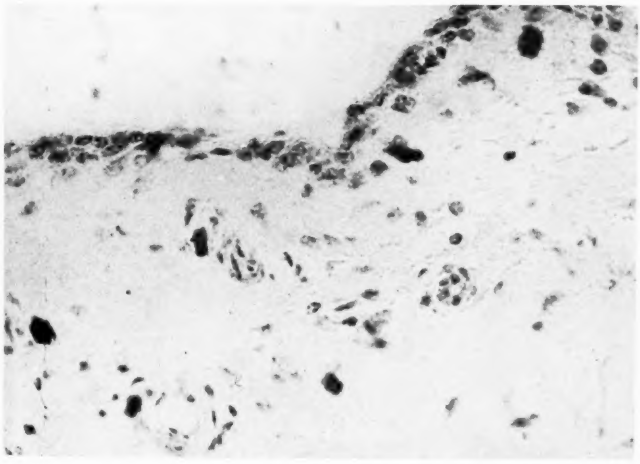

Fig. 2

Fig. 4

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EXPLANATION OF PLATE 1

Fig. 1. Section of the synovial membrane from the knee joint of an ox. Stained with Southgate's mucicarmine. ×400.

Fig. 2. Section of the synovial membrane from the knee joint of an ox. Stained with toluidine blue. The largest dark cells in the subsynovial tissue were stained metachromatically. ×400.

Fig. 3. Sagittal section of the patella and anterior part of the knee joint of a young rabbit. Stained with polychrome methylene blue. The dark area of synovial membrane above the patella was stained metachromatically. ×12.

Fig. 4. Section of the synovial membrane from the knee joint of an ox. Stained by Bensley and Cowdry's method. The relatively large cell in the centre of the field shows mitochondria. (The remaining cells are out of focus.) ×1000.

VARIATIONS IN THE OSSIFICATION OF THE BONES OF THE HAND

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Ossification of the bones of the hand in the normal individual proceed with the regularity which is recorded in most anatomical text-books. There are, however, certain variations, the cause and the frequency of which appear to have given rise to differences of opinion. They concern for the most part the development of the so-called pseudo-epiphyses and the true supernumerary epiphyses, the latter being regarded as developments from a separate centre of ossification and the former as a bony extension from the diaphysis into its cartilaginous extremity. For the purpose of this study I have examined the radiographs of the hands of 1000 apparently normal infants of ages ranging up to 3 years of age who were sent to the Carnegie Infant Welfare Centre in Birmingham for the detection of early rachitic changes. I have also examined the radiographs of thirty twins varying in age from 2 to 11 years. To contrast these findings with those in known developmental irregularities and pathological conditions I have examined the radiographs of the following conditions:

	Cases		Cases
Congenital deformities of the hands	100	Cranio-cleido-dysostosis	6
Rickets (infantile, 50; renal, 18)	68	Unusual dystrophies	10
Scurvy	20	Multiple chondromata	5
Hypothyroidism	12	Multiple exostoses	6
Osteogenesis imperfecta	15	Albers-Schönberg's disease	3
True midgets	20	Osteitis fibrosa cystica (hyper-parathyroid)	4
Achondroplasia	11		
Chondro-osteo-dystrophy (Brailsford)	10	Myositis ossificans, progressiva	3

In addition, for the purpose of this paper I have also examined the published radiographs of cases of arachnodactyly and the unusual dystrophies published by Ellis and others.

Of the 1000 apparently healthy normal children eighty-nine showed abnormal ossification of the metacarpal bones. In eighty-three of these the abnormal development might be considered to be of the nature of pseudo-epiphyses; sixty-two were at the proximal extremity of the 2nd metacarpal and twenty-one at the distal end of the 1st metacarpal. The other six showed true supernumerary epiphyses at the proximal extremity of the 2nd metacarpal, with pseudo-epiphyses at the proximal end of the 3rd, 4th and 5th metacarpals and the distal end of the 1st metacarpal.

The appearance of the pseudo-epiphyses varied. In sixty cases bony continuity between the diaphysis and the pseudo-epiphysis was obvious. In the case of the 1st metacarpal the base of the pseudo-epiphysis appeared to be wholly fused with the diaphysis, but in the 2nd metacarpal fusion was present only in the lateral half—a deep cleft separated the inner half. In twenty-three cases of infants under 2 years of age (at the distal end of the 1st metacarpal in ten, and at the proximal end of the 2nd metacarpal in thirteen) there was a round ossicle without any evidence of bony continuity with the diaphysis (see Pl. 1, figs. 1–3). This appearance gave rise to the

suggestion that these were true supernumerary epiphyses, though they appeared and (as subsequent radiographs showed) fused with the diaphyses before the normal epiphyses showed any sign of an ossific nucleus. It may be that a bony bridge existed though it was undetectable by non-screen radiography. Double intensifying screens in casette with films are not used by the author for this type of work because the grain of the screens obliterates the fine detail of the cancellous tissue of infant's bone.

The possibility that these rounded ossicles developed from a separate nucleus cannot be denied. In support of this is the fact that in two cases infantile rickets was present and the diaphysial border adjacent to the nucleus showed some blurring while the epiphysial extremities of the other diaphyses without such nuclei were clearly defined. In two cases the nuclei were of greater density than the diaphyses even when bony continuity was present (as in Pl. 3, fig. 7), and I have shown that some epiphyses, particularly of the terminal phalanges, often exhibit a greater density than the other normal epiphyses, apparently as a variation of the normal; they fuse at the same time as the other epiphyses and become indistinguishable. So marked is the density that one author has misinterpretated it as representing avascular necrosis of the epiphysis. In the condition of multiple chondromata it is the epiphysial extremity of the diaphysis which shows radiographic evidence of chondromatous metaplasia and in two cases of this dystrophy the diaphysial extremity bearing the pseudo-epiphysis showed chondromatous metaplasia.

The impression is created by the radiographic study of these cases that separate additional osseous nuclei may develop in the first and second years of life and fuse before the osseous nuclei for the normal epiphyses appear. In certain dysostoses, as will be seen later, other separate additional osseous nuclei develop soon after the diaphysial nuclei, remain without bony union until puberty, and then unite with the diaphyses a year or so before the normal epiphyses fuse. Posener, Walker & Weddell (1939), examining the radiographs of 100 children between the ages of 4 and 8 years, reported the finding of true double epiphyses in two cases, the supplementary epiphyses in both being at the distal end of the 1st metatarsal. On the other hand, they reported pseudo-epiphyses in 96% of the children's hands. In the earlier age periods of my 1000 cases I found but 8·5%. This increase in percentage in the findings at the later age period, which was also recorded by Stettner (1931), would indicate that, though in a small number of cases the diaphysial extension producing the pseudo-epiphysial appearance can be recognized in the first two years of life, in the majority this development is not recognizable until the later age period, and that though in the earlier age period a separate osseous nuclus is apparent for a time in some cases no evidence of this was observed at the later age period.

It has been noted that the metacarpals of certain mammals, e.g. the whale, walrus and seal, exhibit double epiphyses. This is regarded by certain authorities as indicative of retarded growth, a provision for keeping the elements of the limb more pliable. It is further considered that in the primitive formation a proximal epiphysial nucleus is laid down, but the more accelerated growth of man and animals leads to invasion of the epiphysial area before an independent ossific nucleus can be developed. Siegert (1910), Köhler (1912) and Rochlin (1929) have discussed this theory in relation to infantile myxoedema and mongolism. Josefson (1916) regards the pseudo-epiphysis as a stigma of endocrine disturbance and Köhler considers that their appearance indicates

retardation of skeletal growth due to endocrine insufficiency. The radiographic evidence which I have obtained from my cases appears to cast some doubt on these theories.

Hypothyroidism is certainly a condition in which retardation of growth is a characteristic feature. In this condition evidence of ossific nuclei for the epiphyses is very late in appearing on radiographs—a delay of one or more years may be indicated. In the case of the larger epiphyses such as those for the femora, humeri and tibiae, the ossification proceeds slowly from multiple nuclei. The epiphyses consequently do not present the normal homogeneity and strength of the normal; they are deformed by pressure as I have shown (1935). These appearances have been mistaken for those of osteochondritis, such as are seen in Legg's disease; radiographs and details of cases of hypothyroidism have been published as cases exhibiting osteochondritis in multiple epiphyses. Now, in none of the cases of hypothyroidism which I have examined did the radiograph of the hands reveal any true supernumerary or pseudo-epiphyses.

In the condition of chondro-osteo-dystrophy (Brailsford) in which I described the ossification of each epiphysis as proceeding from multiple irregular ossific nuclei (1929), I found no true or pseudo-epiphyses except in one or two showing a mild type of the dystrophy. The condition of myositis ossificans progressiva is associated with a characteristic stunted development of the big toe, the middle phalanx of the little finger, and sometimes the bones of the thumb, but no true or pseudo-epiphyses were found. Except in a few isolated cases the same was true in the cases of rickets, scurvy, hyperthyroidism, Albers-Schönberg's disease and osteogenesis imperfecta, conditions in which normal ossification is seriously impaired. In a case of generalized polyostotic fibrous dysplasia supernumerary epiphyses were seen in the 2nd metacarpals, which fused with the diaphyses during the ages of 10 and 11 years.

Radiographs of *congenital deformities* of the hands such as polydactyly, hypodactyly, zygodactyly and syndactyly (including Apert's acrocephalosyndactyly), as well as many in which bony elements were missing or fused, did not show either true or pseudo-epiphyses. There were certain other skeletal dysostoses in which true and pseudo-epiphyses occurred so commonly that they should be regarded as a feature in these conditions.

Cranio-cleido-dysostosis has been regarded as a condition in which defective ossification occurs only in those bones which are laid down in membrane. Thus the bones of the skull exhibit great delay in ossification, the fontanelles are large, and the sutures are represented by wide divisions. In these spaces multiple separate ossific nuclei develop—the so-called Wormian bones. In the clavicle one or two ossific nuclei may be laid down, but development is so seriously impaired that even in the adult the clavicles are represented by but small ineffective bony elements. A comprehensive radiographic examination of the whole skeleton reveals signs suggestive of delayed ossification in other bones besides the membrane bones. Even in the adult the neural arches in the upper dorsal and lower lumbar vertebrae may have failed to fuse in the mid-line—a feature to be noted in the young infant's spine. Defective development of the innominate bones and the femoral necks is also seen.

I have shown (1935) that the bones of the hand in this condition present radiographic appearances which are very suggestive, if not characteristic (see Pl. 1, fig. 4). The terminal phalanges do not develop the prominent cancellous tuft as in the normal phalanx; instead, these phalanges tend to taper from their bases to their free extremities. Later they may be surmounted by a slightly expanded and rounded head.

The middle and proximal phalanges of the fingers have additional epiphyses at their distal extremities which fuse with the diaphyses usually before the age of 4 years, those at the distal end of the middle phalanx of the little fingers being the last to fuse. The proximal epiphysis at the base of certain phalanges, notably the middle phalanx of the index and little fingers, and the proximal phalanx of the 1st and 5th toes, are less marked; the distal phalanges of the index, middle and little fingers exhibit an unusual development. From the distal surface of the proximal epiphyses a cone-shaped prolongation grows towards the diaphysis and is eventually surrounded by the expanded base of the diaphysis. In the case of the little finger and toe, irregular development of the diaphysis of the middle and proximal phalanx sometimes results in bending and shortening of the involved finger or toe. The terminal phalanges of the big toe and thumb appear to be developed from two nuclei, the proximal representing the epiphysis, being in the case of the big toe the larger, and in the case of the thumb as large as the distal diaphysial element. This development of both phalanges of the great toe leads to a stunting in the growth of this digit. These irregularities in growth are associated with supernumerary epiphyses at the proximal ends of the 2nd and 5th metacarpals. These show the characteristic features of the base of the normal diaphysis and contribute to make the metacarpal of normal length. Though they do not fuse with the diaphyses until puberty, they fuse long before the normal epiphyses of the phalanges and metacarpals have united with the diaphyses.

There is a dysostosis which often affects only the long bones of the hands and feet. In mild cases only one or more of the phalanges may show evidence of it, and this evidence is only secured accidentally by radiography of the part in search for fractures. In cases presenting severe degrees of this condition the growth of the long bones of the hands and feet is so seriously affected that evidence of the dysostosis is detected clinically by the unusual shortness and some bending of the involved fingers. The bones of the forearm and leg may also show evidence of defective growth in such cases. In one case there was bilateral defection growth of the lateral half of the superior tibial epiphyses with lateral displacement of the patellae.

Radiographically (see Pl. 2, fig. 5), the terminal phalanges are seen to be represented by two osseous nuclei, the proximal epiphysial element being larger than the distal diaphysial element. As in cranio-cleido-dysostosis the terminal segment is somewhat cone-shaped, and the tuft of cancellous tissue is not developed. From the distal surface of the epiphysial segment a slender cone-shaped prolongation grows into the base of the diaphysis. The latter, at first indented at the site of contact, later appears to expand to accommodate the epiphysial extension. The changes are most marked in the middle phalanges which also show evidence of fused distal epiphyses. In these phalanges there is complete envelopment of the cone-shaped epiphysis by the expanded sheath-like base of the diaphysis, resulting in marked shortening of the phalanx. In some cases the growth of this diaphysial sheath is irregular; only one side appears to develop, the joint surface becomes oblique, and as a result there is unusual bending of the shortened finger. The impression is given in a few cases that the two epiphysial elements fuse to form a central core with the diaphysial sheath wholly or partly surrounding it. The proximal phalanges show distal epiphyses which fuse at an early age, and proximal cone-shaped epiphyses with their tapered extremity enveloped by the base of the diaphysis. The distal epiphyses of the metacarpals show similar but less well-developed characters. Supernumerary epiphyses are seen in the 1st, 2nd and

5th metacarpals which, as in cranio-cleido-dysostosis, retain their identity until puberty and then fuse before the normal epiphyses do.

In the condition of arachnodactyly true supernumerary epiphyses are to be seen in the phalanges and metacarpals of the long slender fingers. In the very unusual dystrophy described by Ellis (1934), which has been referred to as an example of Albers-Schönberg's disease (marble bones or osteopetrosis), though it does not present the characteristic radiographic features of this disease, well-marked extra epiphyses were shown at the proximal extremities of the 2nd and 5th metacarpals and all metatarsals.

In that type of multiple chondromata in which the phalanges and metacarpals are involved, and which includes Ollier's dystrophy, the chondromatous dysplasia affects the diaphysial extremities from which growth is most rapidly proceeding and which bear the epiphyses. In two of the five cases which I examined for the present study extra epiphyses were present on the 1st and 2nd metacarpals, and the distal extremity of the diaphysis of the 1st metacarpals exhibited chondromatous irregularity. In another unusual dystrophy, which affected all the epiphyses of the body and was ultimately associated with a severe degree of scoliosis and bilateral coxa vara, radiographs of the hand at the age of 5 years (23 May 1935) showed all the epiphyses for the long bones except the terminal phalanges of the fingers and the proximal extremity of the 1st metacarpal. Most of these epiphysial nuclei were laid down in much denser bone than normal. By 19 April 1937 no further epiphysial nuclei had appeared, though those existing had grown and those which were dense had become denser. By 22 October 1940 (see Pl. 3, fig. 7) the proximal epiphyses of the proximal phalanges on the left hand had fused with the diaphyses, but those for the index and middle fingers had lost their density and exhibited the normal cancellous structure of the diaphyses. On the right hand the epiphyses for the ring and little finger had fused and lost their density, but those for the index and middle fingers retained their density and separate entity. A minute proximal ossific nucleus representing the epiphysis for the 1st metacarpal had appeared but none were yet visible for the terminal phalanges of the fingers. By 17 June 1942, the patient being now 12 years of age, all the epiphyses previously shown, with the exception of those at the proximal extremity of the 1st metacarpals and some smaller ones which had appeared at the distal extremity of these metacarpals, had completely fused with the diaphyses, and only the epiphyses at the distal extremity of the 2nd and 3rd metacarpals on both hands and that on the proximal extremity of the proximal phalanx of the right hand had retained the unusual density. It was apparent that this, too, was becoming less. No epiphyses have appeared on the terminal phalanges of the fingers of either hand. All the larger epiphyses of the skeleton have also fused.

Examination of the radiographs of the hands of true midgets varying in age from 18 to 36 revealed that four of the twenty had supernumerary epiphyses for the 1st, 2nd and 5th metacarpals. In one male patient aged 22, who was 2 ft. 11½ in. in height, none of the normal epiphyses of the hand or wrist or the supernumerary epiphyses at the proximal end of the 2nd metacarpals showed any evidence of bony union, and though the extra epiphyses of the 1st and 5th metacarpals had fused, the line of fusion was still obvious (see Pl. 2, fig. 6).

Of the eleven cases of achrondroplasia four showed supernumerary epiphyses of the 1st and 2nd metacarpals.

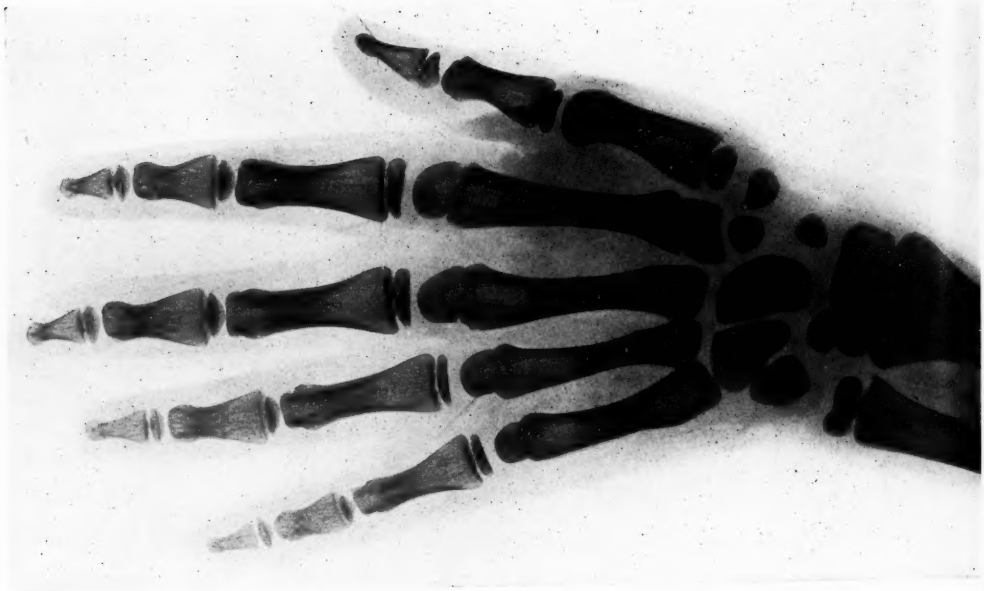
In one case at the age of 1 year no ossific nuclei for epiphyses could be seen on the

Fig. 1

Fig. 2

Fig. 3

Fig. 4



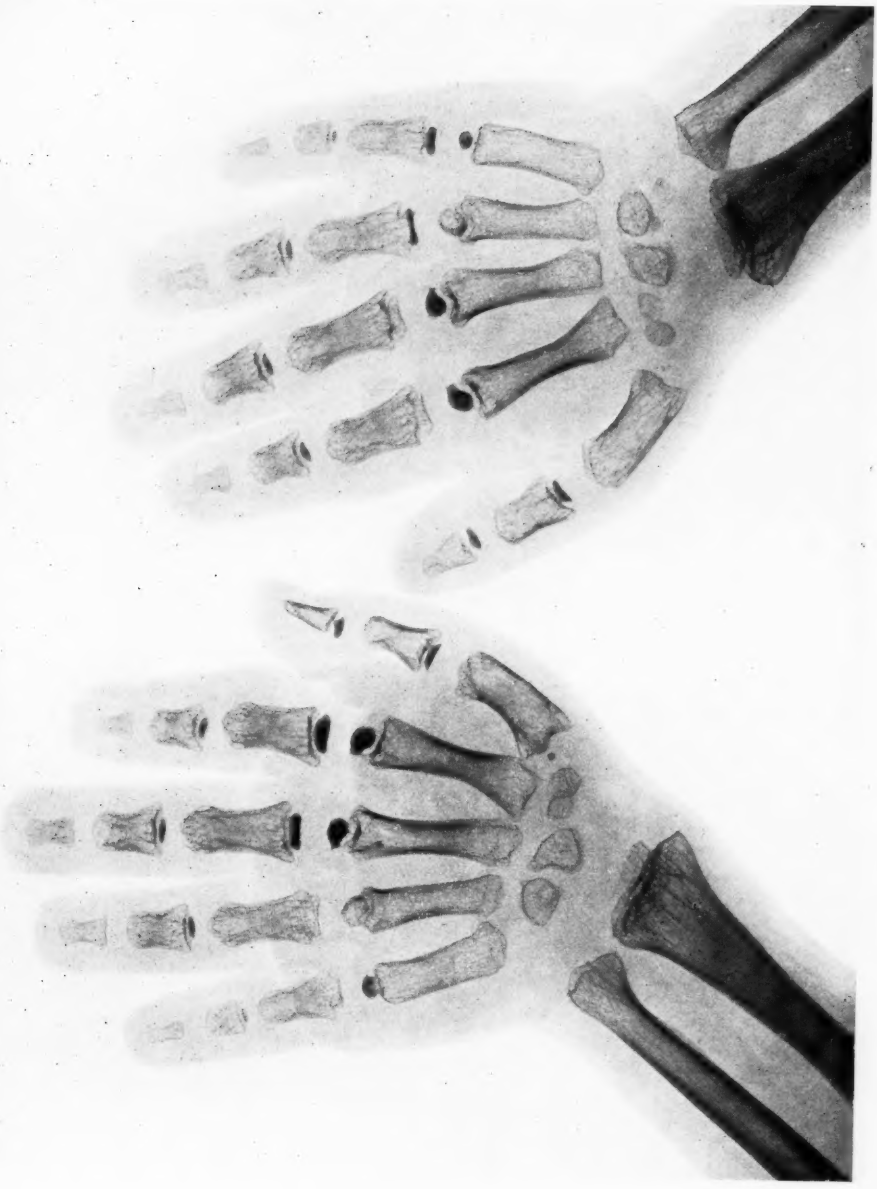


Fig. 7

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radiographs, but at the age of 6 years all the normal epiphyses were present as well as supernumerary epiphyses for the 1st and 2nd metacarpals, the latter having the appearance of commencing fusion.

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EXPLANATION OF PLATES 1–3

PLATE 1

Fig. 1. H. D., aged 18 months. Note separate ossicle at base of 2nd metacarpal.
Fig. 2. J. C., aged 9 months. Note separate ossicle at base of 2nd metacarpal.
Fig. 3. J. H., aged 9 months. Note separate ossicle at base of 2nd metacarpal and a prolongation of the base of the shaft of the bone towards it.
Fig. 4. D. B., aged 10 years. Cranio-cleido-dysostosis showing supernumerary epiphyses.

PLATE 2

Fig. 5. J. R., aged 8 years. Digital dysostosis. Note unusual ossification of phalanges and supernumerary epiphyses.
Fig. 6. R. S., aged 22 years; height 2 ft. 11½ in. True midget showing supernumerary epiphyses.

PLATE 3

Fig. 7. J. C., aged 10 years. Rare dystrophy showing unusual and early ossification of epiphyses.

A COMPLETE COMMON PARASEPTAL CARTILAGE IN A PRIMATE

By E. FAWCETT*, *Department of Anatomy, University of Bristol*

So far as I know, the occurrence of a complete paraseptal cartilage has never been described in a primate. By the term 'complete paraseptal cartilage' I mean a cartilage formed along the nasal septum by the union of the anterior and posterior paraseptal cartilages. A complete lamina transversalis anterior connecting the anterior paraseptal cartilage or the septum nasi with the lateral cartilaginous wall of the nasal capsule has been described in several of the South American monkeys, e.g. *Chrysothrix*, *Mycetes*, *Cebus*, and *Ateleus*, under the name crus communicans. The very complicated description by Frets (1913) may be reduced to the statement that 'bei allen Föten findet sich eine Verbindung des Processus lateralis posterior superior mit dem Processus navicularis ein Crus communicans. Bei *Mycetes B.* ist es gut ausgebildet, bei *Mycetes B.* findet sich bloss Berührung, bei *Mycetes*? und *Mycetes A.* fehlt es. Bei *Ateles* ist das Crus communicans gross, mehr eine Lamina communicans.'

The specimen concerning which this communication is written was destroyed during the course of an air raid. Fortunately, the reconstruction model remains, but all the sections, drawings, and notes made for publication were destroyed, so that while it is possible to redraw the model it is not possible to submit any drawn microscopic sections of the region.

Description of the model. This model represents, at a magnification of 100, the nasal capsule of *Mycetes seniculus*, probably at the 25 mm. stage, for the vomer is still double and of small size with no hint of union. It is very like the vomer of the human embryo at the 30 mm. stage. When the nasal capsule is viewed from below, it is seen to consist of a central part, the septum nasi, which posteriorly widens out to become the presphenoidal part of the central stem of the basis cranii. Springing from the upper edge of the septum are the two halves of the lateral nasal capsule. As Fig. 1 shows, each lateral part is divisible into three segments, an anterior, a middle, and a posterior, the divisions being effected by well-marked sulci; thus, an antero-lateral sulcus separates the anterior segment from the middle segment, and a postero-lateral sulcus separates the middle segment from the posterior one. At the anterior extremity of the anterior segment is the anterior cupula (*C.N.A.*), i.e. cupula nasalis anterior. At the posterior extremity of the posterior segment is the cupula nasalis posterior. From the middle of the lower free edge of the anterior segment (pars anterior) a cylindrical rod of cartilage, the lamina transversalis anterior (crus communicans of Frets), descends to near the septum where the latter is changing from a more vertical to a more horizontal direction. On nearing the septum, the lamina transversalis anterior splits to allow the passage of the duct of the organ of Jacobson to descend to the anterior palatine canal. The dorsal limb is short and ends freely behind; the more ventral limb joins the anterior end of the common paraseptal cartilage.

* Since this, his last scientific contribution, was received for publication, the death of Prof. Fawcett was announced. An obituary notice will be found on p. 192.

The common paraseptal cartilage runs backwards infero-lateral to the septum nasi, and for half its length it runs parallel with the septum. In this part of its course the common paraseptal cartilage is of some depth, as the text-figure shows (*C.P.C.*), but it begins to turn away from the septum and at the same time becomes cylindrical and much smaller than in the anterior half. Opposite and medial to this narrow segment is a half of the vomer which, at this stage consisting of two halves, seems to be more of a covering bone to the paraseptal cartilages than to the septum, in the same way as the paraseptal process of the os incisivum is a covering bone to the anterior segment of the common paraseptal cartilage, or, where a common cartilage fails, to the anterior

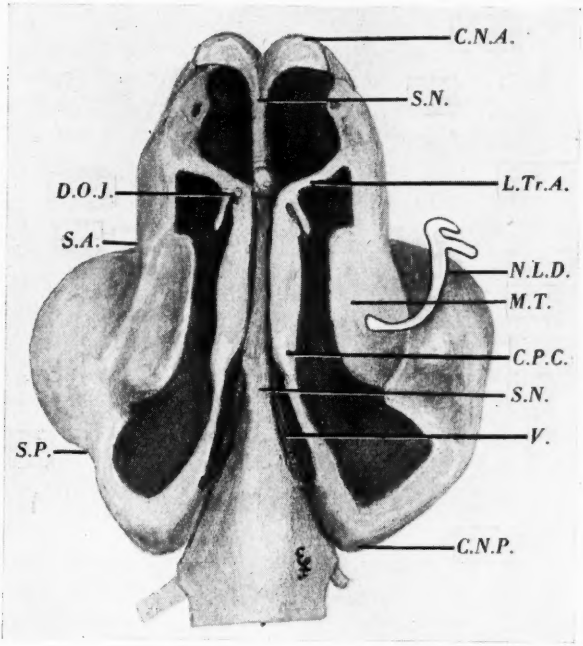

Fig. 1. Inferior aspect of nasal capsule of foetus (*c.* 25 mm.) of *Mycetes seniculus*. *C.N.A.* cupula nasalis anterior; *C.N.P.* cupula nasalis posterior; *C.P.C.* cartilago paraseptalis communis; *D.O.J.* duct. org. Jacobson; *L.Tr.A.* lamina transversalis anterior; *M.T.* cart. maxillo-turbinalis; *N.L.D.* duct. nasi lacrimalis; *S.A.* sulcus antero-lateralis; *S.P.* sulcus postero-lateralis; *S.N.* septum nasi; *V.* vomer.

paraseptal cartilage, as in man. The foetus was stated by the collector to be that of *Mycetes seniculus*, the South American red howling monkey, and as he was a reliable man, there can at least be little doubt that the identification of the genus is correct. Fortunately, also, a model was made of the larynx and trachea showing the enormous enlargement of the upper three rings of the trachea to form a resonating chamber, so that it is certainly the larynx of a howler. The appearance of the model, however, is so different from that drawn by Frets that there is room for some doubt as to the species.

Fig. 1 represents the nasal capsule from below and in front. It should be noted that

the part of the septum nasi shown anterior to the laminae transversales is at right angles to the part shown behind the laminae. The total length of the model viewed in this way is about 12 in., and the drawing is five-twelfths of that length. The sections from which the model was made were cut 20 μ thick and were stained with Victoria blue for the cartilage and acid fuchsin for the bone.

My best thanks are due to Prof. John Beattie for his help in putting literature at my disposal, without which this paper could not have been completed. I should also like to acknowledge the kindness of Prof. Yoffey in providing me with a room in which to do this work.

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A MORPHOLOGICAL STUDY OF TESTICULAR DESCENT

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Much attention has been paid during recent years to the problem of testicular descent with special reference to hormonal influences. It was hoped that studies in this field would have thrown some light on the causation of the changes in position of the testis occurring during human development. But there are two obstacles to the fulfilment of this hope: first, there are gaps in our knowledge of the general topography of the structures concerned with testicular descent and, secondly, though the same biological laws govern man and other mammals, anatomical studies of the latter are rather inconclusive since the structures are not identical in all types. An endeavour has therefore been made to trace the development from their first appearance of the structures concerned with the descent of the testis, using human material only.

HISTORICAL

Similar surveys have been made from time to time for the last one hundred and fifty years. Galen referred to the occasional abdominal position of the testis, and Vesalius described the processus vaginalis, but no work of value was published on testicular descent till that of several men working independently at about the same time. Of these investigators the most important were Haller (*c.* 1750), Camper (1756), Paletta (1777), Vicq d'Azyr (1784), and John Hunter (1786). Hunter's contribution, in particular, is of great value and few of his observations have needed correction. Amongst those who have contributed more recently to our knowledge of the subject are Bramann (1884), Weil (1885), Lockwood (1888), Frankl (1895, 1900), Klaatsch (1896), Felix (1905, 1912), Berry Hart (1909), and Moszkowicz (1935).

MATERIAL AND METHODS

Fifty specimens of human embryos were examined. The methods of examination varied according to the stage of development and the nature of the anatomical detail to be investigated. The younger specimens were stained in bulk with haematoxylin and eosin, and were studied in transverse and longitudinal sections cut at a thickness of 10 μ. Older specimens were mounted on celloidin and dissected, unstained, with or without the aid of a dissecting microscope. The findings obtained by dissection were confirmed by means of serial sections.

The subject is considered in four parts. The first deals with the position of the testis in relation to the abdominal wall and the early stages of development of the plica inguinalis, and includes a study of embryos from 12 to 20 mm. C.R. length; the second part deals with the early development of the gubernaculum and processus vaginalis and other structures which finally take a share in testicular descent, and is concerned with embryos ranging from 20 to 50 mm. C.R. length; the third part deals with the appearance of the structures in specimens from 50 mm. to 23 cm. C.R. length; the fourth part deals with the final stages of descent as seen in specimens of 24 cm. C.R. length and over.

It is realized that the methods outlined above have the limitations that must always exist when vital processes are studied by the use of dead material, and that the mechanical forces at work in bringing about the changes in the relative positions of the various structures concerned can be only suggested and not proved by these methods. It may be presumed, however, that the whole development of this region is organized by the testis under the control of the pituitary gland, though this aspect of testicular descent cannot be considered in a study such as this.

POSITION OF TESTIS AND EARLY DEVELOPMENT OF PLICA INGUINALIS

During the greater part of embryonic life the testis lies at the caudal end of the abdominal cavity. Its position is associated with the development of the infra-umbilical part of the abdominal wall and is determined by the rearrangement of the constituents of the urogenital fold consequent upon the atrophy of the mesonephros.

In an embryo of 14 mm. it is represented by a thickening of germinal epithelium, 1·04 mm. long, lying in the long axis of the body between the suprarenal gland medially and the mesonephros, Wolffian and Müllerian ducts, and abdominal wall laterally.

In 20 mm. embryos the gonads can be displayed by dissection. In one specimen at this stage the right gonad was found to be 2·3 mm. long and the left 2·5 mm. They were situated between the large suprarenal gland and the abdominal wall and ventral to the metanephros. The shortest distance from the gonad to the definitive groin was 0·7 mm. on the right side and 1·0 mm. on the left. These measurements were confirmed by the dissection of other specimens of an approximately similar age.

In a 30 mm. embryo the right gonad was found to be 3·0 mm. in length and the left 2·8 mm. The shortest distance from the groin was 1·6 mm. At this stage the gonads were lying almost horizontally and were separated from the suprarenal gland by the metanephros.

Some writers, e.g. Hunter, Cooper (1845), Cleland (1856), Peters (1927), Keith (1933), and Hamilton Bailey (1936), have suggested that the testis is developed high in the abdomen in a position that would correspond with that of the kidney in the adult and that it then descends to the groin. Thus Peters writes of an 'inneren Descensus'; Keith states that 'The testis descends from the loins to the iliac fossa in the third month'; and Hamilton Bailey embodies in a figure these errors with regard to the early position of the testis.

Although its upper pole does correspond at first with the last thoracic segment, the gonad is never far from the groin. Bramann was the first to point this out, showing that the maximum distance between the testis and the groin in early stages is no more than 1·0 mm. Felix also has denied that there is any abdominal descent.

Though there is certainly a rapid increase in the distance between testis and kidney, this is not due to any downward movement of the testis but is accounted for almost completely by the rapid growth of the lumbar part of the vertebral column and the adjacent structures. This growth carries the metanephros upwards since it is free to slide up behind the peritoneum, while the testis, rendered relatively immovable owing to being suspended by its mesentery, is left attached to the groin. This fact was emphasized by Lockwood, who marked the relation of the testis to the acetabulum as a fixed point and noted that for some time their relative positions remained constant.

If there is any alteration at all in the distance between the testis and the groin it is an increase, as can be seen from the measurements of the embryos in this series given in Table 1. This increase in the distance between the testis and the groin has been referred to by Weil as a 'preliminary ascent'.

The long axis of the gonad is at first parallel to that of the embryo, but quickly assumes an oblique and almost horizontal position; this is due partly to the atrophy of the mesonephros and partly to the increase in size of the suprarenal gland and kidney which apparently pushes the cranial pole of the gonad laterally. Pl. 1, fig. 1 shows the gonad lying parallel to the groin in a 49 mm. embryo.

The urogenital ridge, comprising the gonad, the mesonephros, and the Wolffian and Müllerian ducts, has four peritoneal folds developed in association with it; they are the Wolffian mesentery, the mesorchium, the plica diaphragmatica, and the plica inguinalis.

The Wolffian mesentery, or urogenital mesentery, at first slings all the components of the urogenital ridge to the dorsal wall of the abdomen, and when the mesonephros atrophies it suspends the gonad and the ducts from the dorsal wall, and later becomes the mesentery of the testis and epididymis. The mesorchium slings the gonad to the mesonephros at first and later forms a connexion between testis and epididymis. The

Table 1

Embryo	C.R. length mm.	Gonad mm.	Groin distance mm.	Embryo	C.R. length mm.	Gonad mm.	Groin distance mm.
H 125	20	2·4	1·0	H 385	41	2·5	1·5
H 181	20·6	2·8	1·1	H 35	42	3·0	1·8
H 271	21	3·0	0·7	H 97	47	3·8	1·8
H 408	22	2·3	1·0	H 226	49	4·0	1·8
H 283	30	2·9	1·6	H 65	56	3·3	0·9
H 434	38	3·4	1·5				

cephalic end of the mesonephros extends at first as far as the lower cervical segments. As its caudal part develops the cranial part atrophies but leaves behind it a thin strand that later on extends from the diaphragm to the cranial end of the Wolffian duct; it is known as the plica diaphragmatica. This fold was named by Kölliker (1849) the 'Zwerchfellsband der Urniere'. Lockwood referred to it as the 'plica vascularis', since he believed that it subsequently contains the spermatic vessels.

At its caudal end the mesonephros is attached to the caudal wall of the abdomen (for in early stages there is no true ventral wall caudal to the attachment of the body stalk) by a mesodermal fold which is probably a continuation of the nephrogenic cord, the unsegmented mesoderm in which the mesonephric units develop at a more cranial level. It cannot, however, be said for certain whether this mesodermal fold is derived from the mesoderm of the intermediate cell mass, i.e. the nephrogenic cord, or from the parietal mesoderm, for the attachment is just where these two mesodermal areas become continuous.

When atrophy of the caudal, as of the cranial, end of the mesonephros occurs this mesodermal connexion becomes thinned out and stretches not from the mesonephros but from the Wolffian duct to the caudal wall of the abdomen. Its point of attachment to the duct is where that structure bends forwards towards the cloaca in the lateral part of the genital cord, or, as Bramann describes it, at 'der Stelle an welcher das Vas deferens aus dem Nebenhoden entspringt'.

This mesodermal fold is the plica inguinalis. It can be seen in Pl. 1, figs. 2, 3 and 4, as a fold with a concave upper border lying medial to the umbilical artery and containing the Wolffian duct as that structure passes to join the urogenital sinus. This is the condition in a 12 mm. embryo. At the 14 mm. stage changes have taken place in the lower end of the urogenital ridge; the mesonephros is much smaller; the gonad is well defined and has its own mesentery; the Wolffian duct enters the urogenital sinus posteriorly rather than from the side and thus turns medially out of the plica inguinalis to reach the sinus; the plica inguinalis reaches the body wall to the lateral side of the umbilical artery while the Wolffian duct remains on its medial side, which means that the artery must cross beneath the plica from the lateral to the medial side. Moszkowicz has described an intermediate stage, in a 13·5 mm. embryo, in which the artery ran parallel to the fold and below it. In a 20 mm. embryo (Pl. 2, fig. 5) the plica inguinalis passes from the caudal end of the urogenital fold to the ventral wall of the abdomen. It is 0·42 mm. long and is attached proximally close to the Wolffian and Müllerian ducts at the caudal end of the fold. In a dissection of a 20 mm. embryo the umbilical artery was seen crossing beneath the inguinal fold from the lateral to the medial side, and the connective tissue round the artery was intimately fused with that of the inguinal fold.

As embryos from 30 to 50 mm. are examined the inguinal fold is found to become more cord-like and isolated from surrounding structures and it acquires a mesentery. Its upper end is attached to the Wolffian duct, as has been stated by Curling (1841), Weil, Lockwood, Frankl, Eberth (1904), Felix, and Moszkowicz. On the contrary, it has been described by Meckel (1820), Cooper, Bramann, and Klaatsch as being attached to the lower pole of the testis.

GUBERNACULUM TESTIS AND PROCESSUS VAGINALIS

The gubernaculum testis has been described by many previous workers as arising from the cells contained within the plica inguinalis. This fold has been referred to above as passing to the ventral abdominal wall, and it may be noted here that it reaches the body wall at a place where there are no muscle fibres, that its cells seem to pass in both a cranial and caudal direction in the abdominal wall, and that some of them pass to the base of the genital swelling and to the inner aspect of the thigh. The appearance of the inguinal fold and its contained cells, or, as it may now be called, the gubernaculum, is seen well in a section of a 20 mm. embryo (Pl. 2, fig. 6). The cells of which it is composed can be distinguished by their irregular arrangement and by being more condensed than those of the body wall. As they pass into the abdominal wall they lie between the rudiments of the rectus and oblique muscles of the abdomen, but in some specimens they seem to bring about a disordered arrangement of the oblique muscles, particularly of the transversus and internal oblique. But the cells of which the gubernaculum is composed are present in situ before the abdominal wall and its musculature are formed, and there is no evidence of its cells invading the lymphatic spaces of the body wall, as was described by Berry Hart (1909).

As the gubernaculum of a 20 mm. embryo is traced more caudally it is found to end indefinitely in the region of the genital swelling, not as a single strand but in a cone-shaped manner, the base being the insertion, i.e. it is attached to an area and not to a point. The dissection of the distal end of the gubernaculum in embryos of

12 or 14 mm. is very difficult owing to the loose friable nature of the strands of which it is composed and to the fact that they have no definite borders but merge into the surrounding musculature.

In older embryos dissection of the gubernaculum is easier, for it becomes a more cord-like structure lying in the free edge of the plica inguinalis. When a 20 mm. embryo (Pl. 2, figs. 5, 6) is compared with an older specimen, for example of 42 mm. (Pl. 2, figs. 7, 8), it is found that in the latter the distal end of the gubernaculum appears to invaginate the abdominal wall and thus lies in a small pocket, to the dorsal wall of which it is attached by a short, thick 'mesentery'. This little crescentic pocket is the beginning of the processus vaginalis.

The time at which this processus vaginalis makes its appearance is variable; Weil gives the end of the second month as the usual time; Bramann, and Lockwood, first noted it in the 14th week; Frankl, and Eberth, describe its presence in embryos of 28 mm.; in the present series it can be seen on both sides in an embryo of 40 mm., on one side in one specimen of 42 mm. and absent in another, absent in a female embryo of 47 mm., and present on the right side only in a male embryo of 49 mm. As serial sections of the 42 mm. embryo (Pl. 2, figs. 7, 8) are studied, it is found that though the processus vaginalis appears as a crescentic cleft it is in reality a blind pouch, open at its cranial end into the abdominal cavity, and lined with coelomic epithelium. It appears to surround the gubernaculum on all sides except dorsally, and it separates that structure from the fibres of the cremaster muscle laterally. In this embryo the vaginal process is 0·21 mm. long.

At this stage the gubernaculum contains non-striped muscle fibres which form a large part of its bulk, and as it is traced distally its outline becomes less distinct owing to the intermingling of its fibres with the constituents of the abdominal wall.

The above description differs from that of several previous workers, especially with regard to the relation of the gubernaculum to the processus vaginalis. The latter is said by Weil, Weber (1847), Meckel, Klaatsch, Frankl (1900), and Kirk (1936) to sink into and hollow out the gubernaculum, so that the gubernaculum is divided into two parts, a central core and a superficial part which becomes the cremaster muscle. There is no evidence to support this in the present series. On the other hand, the findings do support the descriptions of Bramann, Hunter, Cooper, and Lockwood, namely, that the processus vaginalis is a sac of peritoneum which develops on the ventral aspect of the gubernaculum and becomes covered with a layer of striated muscle from the internal oblique and transversus muscles, which layer is called the cremaster. The formation of the processus vaginalis is not due to the activity or proliferation of any of its parts, but is secondary to growth changes of the abdominal wall, as described by Felix.

FURTHER DEVELOPMENT OF GUBERNACULUM PRIOR TO TESTICULAR DESCENT. EMBRYOS OF 50 MM. TO 23 CM.

The position and relationships of the testis at the commencement of this period differ little from what is found at earlier stages.

The distance from the groin is approximately the same, though the marked angularity at the point of junction of epididymis and vas deferens gives the impression of downward traction.

The processus vaginalis keeps pace with the growth of the whole embryo; it is

1·8 mm. long in a 56 mm. embryo and 3·0 mm. long in an embryo of 120 mm. The inguinal canal is well defined in a 66 mm. embryo; it contains the processus vaginalis, the gubernaculum and the cremaster; its relations are as in the adult, but the deep inguinal ring is not well defined. Keith has denied the existence of an inguinal canal at this stage. There is no evidence of an ascending portion of the cremaster muscle as was described by Hunter, Lockwood, Eberth, and Kölliker.

The testis lies anterior to the epididymis to which it is joined by the mesorchium which gradually becomes shorter. The junction of epididymis and vas deferens is sometimes found at the mouth of the processus vaginalis. The gubernaculum increases only slightly in length during the period under discussion, but it attains a much greater bulk particularly in the later stages, e.g. in embryos of 20 cm. The inguinal canal also increases in width, due almost entirely to enlargement of the cremaster.

In all embryos examined from the 6 to the 12 cm. stage the gubernaculum could be traced to the scrotum and in some cases to the pubic region and to the inguinal ligament also, but no fibres were found going to the perineum. During this period there are practically no striated muscle fibres in the gubernaculum, but they become prominent in later stages, at about 22 cm.

Towards the completion of the development of the parts concerned with testicular descent, i.e. in embryos of about 20 cm., one of the most outstanding features is the great bulk of the gubernaculum so that the testis has the appearance of a little knob perched on its summit; in fact, the growth of the gubernaculum is so great at this stage that it pushes the testis for several millimetres into the abdomen. This growth of the gubernaculum begins at the cephalic end and finally involves the whole structure; it is an intrinsic property of the gubernaculum and is independent of the growth of the cremaster. In none of the present specimens was there found an appearance such as Keith describes of the gubernaculum growing down as a solid cellular mass, with an actively growing cellular cap, into the subcutaneous tissue of the scrotum.

All observers agree that the gubernaculum does come to contain striated muscle fibres in later stages, and these are usually described as being situated peripherally in the gubernaculum and as being cremasteric in origin. Bramann has described a central core of connective tissue as being present in the gubernaculum, while van den Broek (1906) and others have compared the whole structure with the conus inguinalis of some lower forms.

In the present series sections of a 22 cm. foetus show that below the level of the processus vaginalis there is an intermingling of gubernaculum and cremaster, but there is no evidence of any ascending cremasteric fibres contributing to the more proximal part of the gubernaculum. In these sections of the 22 cm. specimen, shown in Pl. 3, figs. 9, 10 and 11, three histological entities are found, striated muscle fibres, unstriated muscle fibres, and blood vessels. The striated muscle fibres, which are well seen in Pl. 3, fig. 11, lie with their long axis parallel to that of the gubernaculum and in the section they are cut obliquely. They grow from the scattered spindle-shaped cells which are found throughout the gubernaculum prior to this stage. The unstriated muscle fibres can be seen lying between the striated fibres. The blood vessels are numerous and tortuous (Pl. 3, figs. 9, 10) and the great vascularity of the gubernaculum is characteristic of this stage, as noted by Weil.

As in the earlier stages, so towards the end of this period, the processus vaginalis keeps pace with the general growth of the foetus. It measures 9·9 mm. in length in

a foetus of 20 cm. It is a flattened sac lying between the gubernaculum dorsally and the cremaster ventrally, and it is only at its cephalic end that it surrounds the gubernaculum completely, thus giving it a sort of mesentery. At this stage the vaginal process is found to be 10 mm. in advance of the testis in the inguinal canal, but this distance seems to be a very variable one. As can be seen from Table 2, the testis doubles its size between the 12 and 20 cm. stages. Its distance from the groin varies.

Table 2. *Statistics of the dissected embryos*

Embryo	C.R. length mm.	Gonad mm.	Groin distance mm.	Guber-naculum mm.	Infra-umbilical belly wall mm.	Processus vaginalis mm.
125	20	2·4	1·0	1·8	1·4	—
181	20·6	2·8	1·1	1·9	1·6	—
271	21	3·0	0·7	1·4	1·6	—
408	22	2·3	1·0	1·8	2·0	—
283	30	2·9	1·6	3·2	3·1	—
434	38	3·4	1·5	3·5	5·2	—
385	41	2·5	1·5	3·5	6·2	0·4
35	42	3·0	1·8	4·0	6·8	—
97	47	3·8	1·8	4·4	7·1	—
226	49	4·0	1·8	3·8	7·2	0·9
65	56	3·3	0·9	4·2	8·0	1·8
219	66	4·0	1·2	6·6	11·0	2·4
134	90	4·4	1·3	6·6	15·6	3·3
388	94	3·8	1·5	5·8	16·0	—
472	118	3·2	2·8	5·9	18·0	2·6
310	137	7·0	--	7·5	20·0	—
150	145	6·6	3·4	13·2	26·0	8·9 × 3·0
528	195	6·4	—	16·0	28·0	9·9 × 5·1
—	220	7·0	6·5	21·0	34·5	11·5 × 4·5
—	230	9·0	2·0	27·5	36·0	12·5 × 5·0
—	240	10·9	—	4·9	46·0	25·0 × 8·5
—	250	10·0	—	1·9	—	24·5
—	260	11·0	—	—	44·0	21·2
—	270	12·3	—	0·7	42·0	28·8 × 5·6

THE ANATOMY OF FINAL DESCENT. FOETUSES OF OVER 24 CM.

In no specimen of 23 cm. or less had descent begun, yet it was completed in every specimen, except one, of 24 cm. and over. Thus in the present series descent of the testis from the groin to the scrotum occurs at the beginning of the seventh month of intra-uterine life. Only normal material was used, and the scarcity of material in which intermediate stages of descent could be studied suggests that the process of descent is a rapid one.

The time at which the mouth of the processus vaginalis becomes closed is a very variable one. In twelve cases where both testes were in the scrotum the processus vaginalis was patent on each side. Seiler (1817), Klaatsch, Frankl, and Moszkowicz have expressed the opinion that the structure which fills the inguinal canal prior to descent is the conus inguinalis which, by the contraction of its own musculature, shortens and turns itself inside out like the finger of a glove, thus forming a cavity which is lined with peritoneum. This conus inguinalis supplants the gubernaculum in the plica inguinalis, leaving only a core of embryonic connective tissue and striated muscle. As has been stated above, the structure that fills the inguinal canal is not the equivalent of the conus inguinalis.

Frankl (1900) and Broman (1911) have shown that descent is accompanied by a shortening of the gubernaculum. This finding is supported by measurements in the

present specimens, in which it was found that the distance between the testis and the bottom of the processus vaginalis remains approximately constant. In every case the main attachment of the gubernaculum was to the bottom of the scrotum but was rather loose, so that it was easily torn in dissection. In one case there was an additional attachment to the pubis. By the time that the testis has reached the bottom of the scrotum there is no sign of the gubernaculum.

Factors causing descent. It is outside the scope of this paper to discuss the reasons why testicular descent does occur in practically all placental mammals. Hunter suggested that the testis was in some way responsible for organizing its own descent, and modern endocrinological research, according to Engle (1932) and others dealing with the subject of cryptorchidism, supports this idea. At the same time Hunter admitted that the causes were probably mechanical. It seems likely that atrophy of the mesonephros, the rearrangement of the structures on the posterior abdominal wall, the development of the infra-umbilical portion of the abdominal wall and the descent of the testis are all closely related.

Cleland, Kölliker, and Berry Hart suggested that descent could be accounted for by differences in the rate of growth of structures related to the testis. Berry Hart considered also that the inguinal canal grew up to envelop the testis. Measurements made on the present series do not at all support this conception. Descent seems to take only a short time, and just before it occurs the testis is 3 cm. from its final position, a distance too great to be covered rapidly by any upward growth of the inguinal canal. Meckel suggested that the testis is pulled down by scar contraction of the gubernacular connective tissue. But there is very little connective tissue in the gubernaculum.

Furthermore, an objection to any theory of gubernacular contraction is the fact that the attachment of the distal end of the gubernaculum is a very slender one and is made to a mobile area of skin. The attachment of its proximal end in the abdomen is much more secure, and so any contraction would tend to pull the scrotum up rather than the testis down.

The intense vascularity, the increase in size prior to descent, and the fact that the striated muscle fibres of the gubernaculum lie in the plane of the inguinal canal must all have some significance. It should also be noted that the testis becomes more mobile just before descent. It was Bell (1826) who pointed out that the vessels of the testis lengthen so that they become 'elegantly tortuous'. Failure of such lengthening would prevent descent.

Finally, it should be emphasized that the testis has a mesentery and that it is not a retroperitoneal organ like the kidney free to slide down the abdominal wall behind the peritoneum.

SUMMARY

1. An account is given of the development of the structures associated with descent of the testis in embryos ranging in size from 12 mm. to 27 cm. C.R. length.

2. The testis is never far from the groin. Up to the 90 mm. stage the average distance is 1·3 mm. Between the 20 and 50 mm. stage the testis shows a preliminary ascent. It doubles in size between 12 and 20 cm. The final descent is rapid and occurs between the 23 and 24 cm. stages, i.e. early in the seventh month.

3. In a 20 mm. embryo the testis lies vertically, but by the 30 mm. stage it is

almost horizontal, and it is parallel to the groin at 50 mm. These changes are due partly to growth of the suprarenal gland and partly to atrophy of the mesonephros.

4. Atrophy of the mesonephros causes a thinning out of the mesentery which originally slings it. The cranial part becomes the plica diaphragmatica, the middle part forms a part of the mesorchium, and the caudal part forms the plica inguinalis. The Wolffian duct at first runs in the plica inguinalis to the cloaca.

5. The gubernaculum develops in the substance of the plica inguinalis. Its cranial end is attached to the Wolffian duct and not to the testis; its caudal end flares out and is attached to the ventral wall of the abdomen where it spreads up and down, to the groin, to the region of the genital swelling, and to the thigh.

6. The gubernaculum becomes greatly enlarged about the 20 cm. stage. It contains striated and unstriated muscle fibres and is very vascular. The muscle fibres are an intrinsic development, and there is no evidence that the muscles of the abdominal wall, particularly the cremaster, contribute to its formation.

7. At about the 20 cm. stage the gubernaculum becomes so large that it pushes the testis for some distance into the abdomen. The gubernaculum does shorten during testicular descent, but there is no evidence that it pushes down as a cap into the scrotum.

8. The processus vaginalis can be seen at the 42 mm. stage as a small crescentic slit 0·21 mm. in length. It passes down the inguinal canal, which is well defined at 66 mm., between the gubernaculum dorsally and the cremaster ventrally.

9. The present study does not support the view that the inguinal canal grows back towards the testis. The flimsy attachment of the gubernaculum to the scrotum is an obstacle to it exerting much traction on the testis.

The material has been provided through the courtesy of Professor C. W. Stump, of the Department of Embryology and Histology, the University, Sydney, from a large collection of human embryos possessed by that department.

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EXPLANATION OF PLATES 1–3

PLATE 1

Fig. 1. Dissection of embryo H 226 of 49 mm. ×6. A well-formed pars abdominalis gubernaculi is evident. The interstitial part has also been dissected, the outline being less clearly defined. 1, gonad; 5, gubernaculum; 6, bladder.

Figs. 2–4. A series of transverse sections through the caudal end of the mesonephric ridge of a 12 mm. embryo H 323. ×35. A, Wolffian duct; B, umbilical artery.

PLATE 2

Fig. 5. Transverse section through caudal region of a 20 mm. embryo H 304. ×20. *b*, plica inguinalis; *g*, pubic anlage.

Fig. 6. The same section as above. ×120. Showing the detailed relations of the cells of the definitive gubernaculum to the belly wall.

Fig. 7. Transverse section of a 42 mm. embryo H 42. ×11. *a*, testis; *b*, gubernaculum.

Fig. 8. Transverse section of a 42 mm. embryo H 42. ×100. *c*, cremaster muscle; *d*, processus vaginalis.

PLATE 3

Figs. 9–11. Sections through the gubernaculum of a 22 cm. foetus. The magnifications are 120, 250 and 550 respectively.

Fig. 1

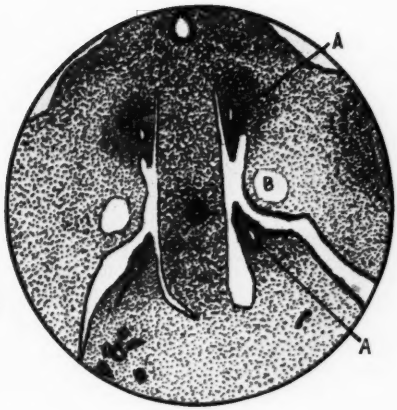

Fig. 3

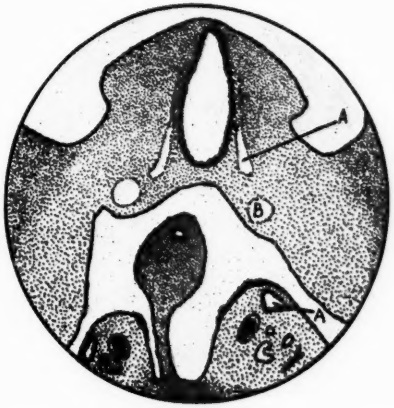

Fig. 2

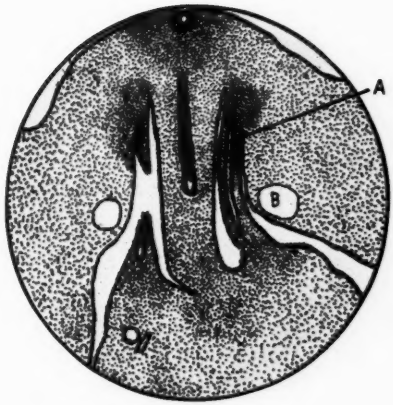

Fig. 4

Fig. 5

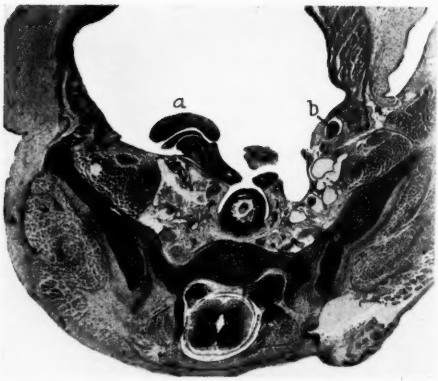

Fig. 7

Fig. 6

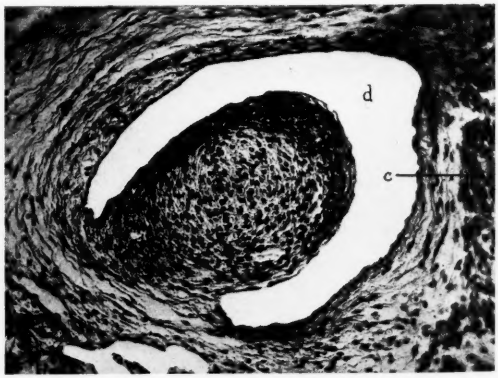

Fig. 8

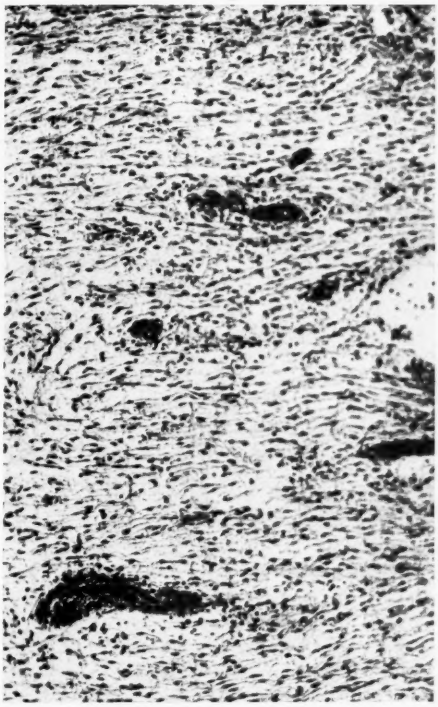

Fig. 9

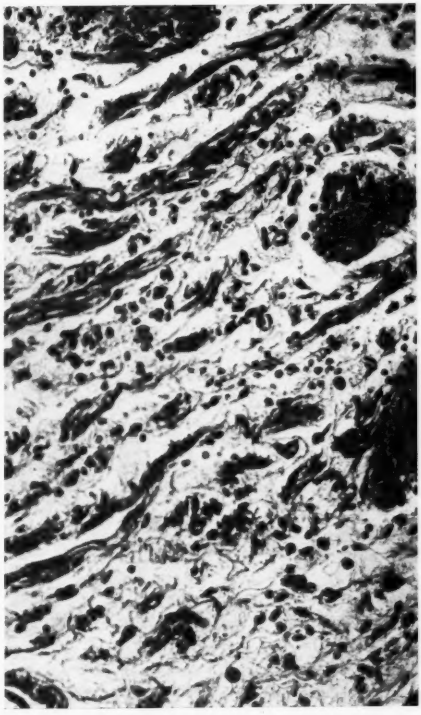

Fig. 10

Fig. 11

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IN MEMORIAM

DAVID WATERSTON, M.A., M.D., F.R.C.S.Ed., F.R.S.E.

David Waterston, Bute Professor of Anatomy in the University of St Andrews, died at Edinburgh on 4 September 1942, aged 71. His death removes an anatomist, trained in the Edinburgh tradition, who contributed freely to several branches of his subject; and it deprives the James Mackenzie Institute at St Andrews of an original member and its chairman.

Waterston, born at Glasgow in 1871, was a son of the manse, his father, the Rev. Richard Waterston, having been Free Church minister at Dundee for many years. He received his University education at Edinburgh, taking the M.A. degree in 1891 and graduating M.B., C.M. with honours in 1895. After a resident surgical post in the Royal Infirmary, he was appointed Demonstrator of Anatomy by Sir William Turner, and he was Crichton Research Scholar for three years (1899–1902). In 1898 he had become F.R.C.S.Ed., and in 1900 he proceeded to the M.D. degree, receiving a gold medal for his thesis on 'Studies in the osteology of the human foetus and infant'. In 1903 he succeeded the late Prof. David Hepburn as Senior Demonstrator and Lecturer in Regional Anatomy under Prof. Cunningham. He thus had the advantage of association with both Turner and Cunningham.

In 1909 Waterston was elected Professor of Anatomy at King's College, London; but his tenure of the chair was a short one, as he was recalled to Scotland in 1914 to succeed Musgrove as Bute Professor at St Andrews. In the attractive environment of Scotland's oldest University he lived a busy and a happy life for twenty-eight years, developing both the teaching and research sides of his Department and extending it by the addition of a model Dissecting Room (fortunately only slightly damaged by recent enemy action). During these years too he had a full share of administrative duties: he represented the Senatus on the University Court for six years and the University on the General Medical Council for ten (1930–40). He was also for a time a member of the Dental Board of the United Kingdom, and lately he had added municipal work to his other activities, having joined the Town Council of St Andrews as a co-opted member.

Prof. Waterston's early contributions to anatomical literature were directly influenced by his association with Sir William Turner, as they were in the fields of physical anthropology and, in collaboration with Hepburn, of cetacean anatomy. Before he left Edinburgh he had edited the *Edinburgh Stereoscopic Atlas of Anatomy* (1905)—a valuable topographical series, long out of print and now almost unobtainable—and had shared with A. C. Geddes (now Lord Geddes) in the preparation of a 'Report on the anatomy and embryology of penguins collected by the Scottish National Antarctic Expedition' (*Trans. Roy. Soc. Edinb.* 1909).

At King's College Waterston's work took a decidedly embryological turn. There he had succeeded the late Prof. Peter Thompson, well known for his reconstructions of embryos by the Born wax-plate method, the technique of which he had brought from Freiburg to King's College in 1906. Prof. Waterston became equally enthusiastic and spent much time in the making of models to illustrate his papers and for use in

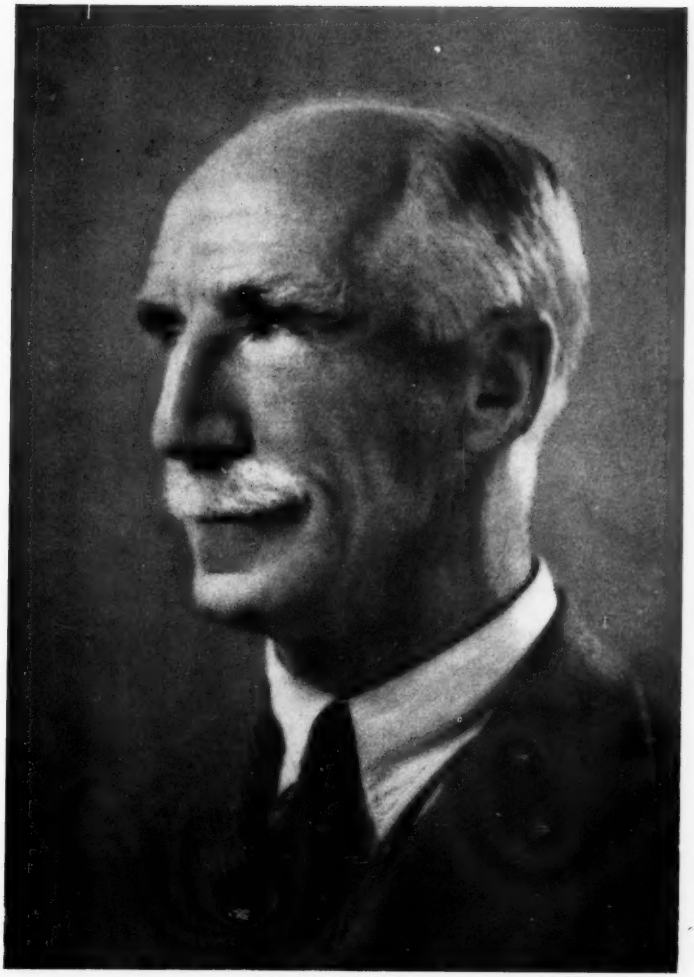
teaching. He described a 'Human embryo of 27 pairs of somites' (*J. Anat.* 1914)—slightly older than Peter Thompson's 2·5 mm. embryo with 25 pairs—and wrote also on the development of the pericardium and pleural sacs (*J. Anat.* 1915). In his last year at King's College he was Arris and Gale Lecturer of the Royal College of Surgeons and delivered a lecture on 'Development of the heart in Man in relation to its functional activity' (*Lancet*, 1914).

At St Andrews Prof. Waterston continued his embryological work and published two main papers—'Development of the heart in Man' (*Trans. Roy. Soc. Edinb.* 1918) and 'Development of the hypophysis cerebri in Man' (*ibid.* 1926). He made contributions also to the technique of reconstruction from serial sections and devised a simple method of making negative prints direct on photographic paper not only to replace the usual time-consuming drawings but to provide a convenient record of the serial sections of an embryo (*J. Anat.* 1923). In 1927 he gave the Struthers Lecture of the Royal College of Surgeons of Edinburgh on 'The comparative anatomy and development of the heart and alimentary canal' (*Edinb. Med. J.* 1927).

Since the founding of the St Andrews Institute for Clinical Research in 1919, however, Prof. Waterston's attention had been directed mainly to the anatomical problems of cutaneous sensation. He was an original member of the Institute and worked in harmony and friendship with Sir James Mackenzie to make this great experiment a success, taking up with enthusiasm investigations likely to further its aim. From 1928 he was a member of the Council and since 1938 he had been Chairman of the Institute. His papers on 'Observations upon cutaneous sensations' and 'Sensory activities of the skin for touch and temperature' (reprinted from *Brain*, 1923) appeared in vol. I (1922) and vol. II (1924) of the Reports of the Institute, and he edited vol. III (1926). In 1923 he contributed to the *Journal of Physiology* a paper in which he described experiments on himself to elucidate the relation of nerve-endings to touch and pain; and at the Bournemouth Annual Meeting of the British Medical Association he opened a discussion on 'Pain and the mechanism of its production' (*Brit. Med. J.* Dec. 1934). Sir James Mackenzie, who suffered from angina, had expressed the wish that a detailed report on the state of his heart should be prepared, and this task was adequately discharged by Prof. Waterston (*Brit. Heart J.* 1939).

During these years Prof. Waterston had become much interested also in local archaeological investigations. He was for a time President of the St Andrews branch of the League of Prehistorians, and he succeeded in interesting his students and in stimulating them to the collection of material. He contributed a number of papers on cists and their contents to the *Proceedings of the Society of Antiquaries of Scotland*, and his last contribution of this kind awaited publication at the time of his death. When the University Chapel at St Andrews was renovated in 1930, Prof. Waterston was asked to prepare an anthropological report on the remains of Bishop Kennedy (1408–65) while a new casket to receive them was being made. His report includes an interesting essay in the reconstruction of a portrait from the features of the skull (*Trans. Roy. Soc. Edinb.* 1934).

Prof. Waterston was an able exponent of his subject, with a reputation for precision of manner and statement in his presentation of it to his students. From his earliest teaching days he took great pains in the preparation of finished blackboard drawings, and his skill as a draughtsman is well seen in the topographical plates (on a basis of orthogonal projection) that illustrate his *Anatomy in the Living Model* (1931).



DAVID WATERSTON
1871–1942

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The opinion has often been expressed, however, that the best part of that book, in conformity with its title, is the chapter on the functional anatomy of the skin. He was a contributor also to Cunningham's *Text-Book of Anatomy*, having been responsible for the 'Digestive System' in successive editions since 1913.

No notice of David Waterston would be complete without reference to his favourite recreation on the links. Readers of this *Journal* who also are golfers may like to be reminded or to know that he contributed a brief article on 'Anatomy and the golf swing' to *Blackwood's Magazine* (1929); and they will not be surprised to learn that it concluded with the traditional advice to 'Keep your eye on the ball'. Whether the author profited directly by his anatomical analysis would be difficult to decide; but he was certainly a good golfer, a member of the Royal and Ancient, and a familiar figure on the old Course at St Andrews.

Prof. Waterston leaves a widow and three sons, two of whom qualified in medicine and are now both in the R.A.M.C. on active service.

J. C. B.

IN MEMORIAM

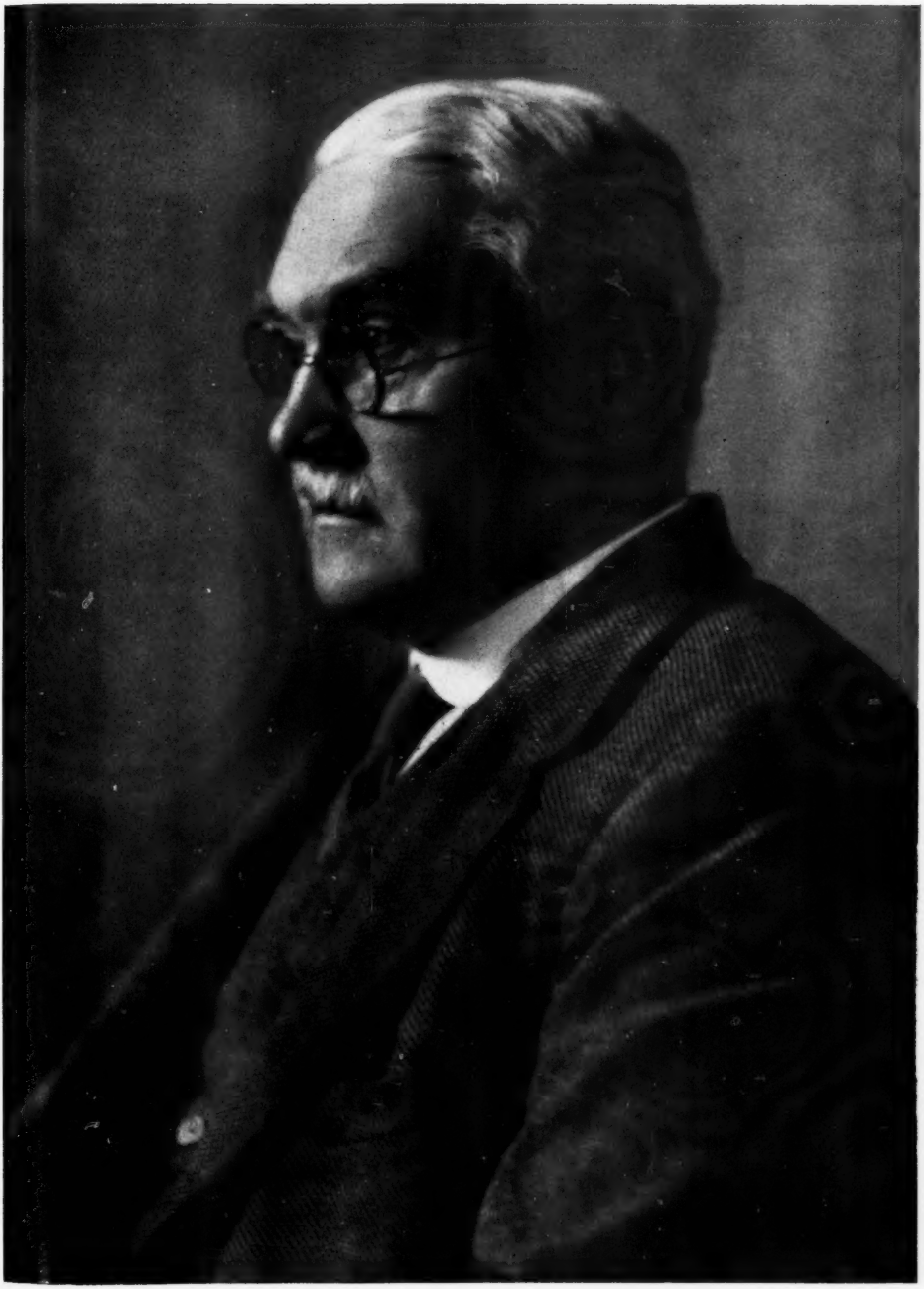
EDWARD FAWCETT, F.R.S., M.D., C.M. (EDINB.)
Emeritus Professor of Anatomy, University of Bristol
1867–1942

In the passing of Edward Fawcett, Anatomy in this country has lost another of its outstanding personalities. It is some time now since he took an active part in anatomical work, but a generation ago he was in the forefront of anatomical investigation, and in the field in which he chose to work he secured international recognition. A fine figure of a man, his carriage was always firm and erect, even in old age, and his bodily vigour was the physical counterpart of a vigorous and active mind.

Born in 1867, he graduated M.B., C.M. at Edinburgh in 1889, and his interest in Anatomy was already manifest even in his student days, for he was Demonstrator in Anatomy in the School of Medicine for two years before he qualified. He spent three years after qualification at Yorkshire College, Leeds, and from there proceeded to Bristol. He was twenty-six years old when, in 1893, he was appointed Professor of Anatomy in University College, Bristol, and he served in that capacity until his retirement in 1934. For a large part of that time (1905–34) he served also as Dean of the Faculty of Medicine. For practically forty years, therefore, he was associated with the Bristol Medical School as a whole, and the Anatomy Department in particular, and in the development of both he took an exceedingly active part. He obtained his Edinburgh M.D. (Thesis Gold Medallist) in 1906, and was elected a Fellow of the Royal Society in 1923.

He was a member of the Anatomical Society of Great Britain from 1891 to 1934 and also held office in the Society, becoming successively Vice-President (1913), Treasurer (1914–25), and President (1927–9). At the Budapest International Medical Congress (1909) he was President of the Anatomical Section. He regularly took part in the proceedings of the Anatomical Society, and contributed many papers to the *Journal of Anatomy*. He demonstrated many beautiful reconstructions (now in the Anatomy Museum at Bristol) at Society meetings, and one of his favourite stories dealt with the difficulty of persuading the railway authorities to transport his fragile specimens with due care.

From a review of an extensive list of published papers, Fawcett's work may be seen to fall, with some overlapping, into four main periods. In the first period he showed a general interest in osteology and arthrology. To this early period belong papers on the morphology of the oblique radio-ulnar ligament (1894), the retinacula of Weilbrecht, mandibular canal, and facets on the talus (1895), varieties of interchondral articulations (1898), and accessory carpal bones (1900). This was followed by a second period, in which his interest was evidently shifting from the morphology of the fully formed bones to problems of bone growth and the formation of epiphyses. To this period belong such papers as those on the ossification of the olecranon (1903), pelvis (1905), ribs (1910), vertebrae and sacrum (1911), and the clavicle (1912). In the third period interest became focused on the development of individual bones of the skull. In this period there appeared papers on the development (in man) of the



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mandible, pterygoid plates and processes (1905), palate bones (1906), sphenoid and chondrocranium (1909), maxilla (1910), and vomer and nasal capsule (1911). These studies led, naturally, to the fourth and most important period, when he published his researches on the growth of the mammalian chondro-cranium.

It is the work done in this fourth period which may be said to constitute Fawcett's most important and fundamental contribution to biological science. From 1917 to 1923 he published in rapid succession detailed accounts of the primordial cranium of a number of mammals—*Microtus* (1917), *Erinaceus* (1918), *Poecilophoca* (1918), *Miniopterus* (1919), *Tatusia* (1921), and *Xerus* (1923). All his work was marked by scrupulous honesty and unremitting attention to detail. Both his histological and reconstruction work were of a uniformly high order. He was a tireless worker, often commencing at 6 a.m., and working until late at night. Perhaps only those of his colleagues who toiled in the same field were really capable of appreciating the enormous amount of work which went into the various reconstructions which he made and described.

However, Fawcett possessed such a superabundance of energy that his anatomical labours, considerable as they were, by no means exhausted it. In 1919 he founded the Bristol Spelaeological Society, and held office as President from its foundation until 1936. He published numerous reports on human skeletal remains submitted to him for examination, and indeed at the time of his death two such reports were awaiting publication—one dealing with remains associated with a beaker at Corston, Somerset, the other with some human bones from a Roman stone coffin exposed during the recent air raids on Bath.

During his latter years Professor Fawcett was engaged on work of an archaeological nature, in which he took great delight. As in his anatomical so in his archaeological work, he was influenced by two factors, the consideration of form and of development. He made a study of the ancient chairs to be found in Bristol and the surrounding counties, and arranged them according to their structural evolution. The same principles made him an accurate student of medieval architecture, for he made detailed observation of mouldings and other architectural features, and so was able to fix the date of a building or part of a building with authority.

He made complete lists of the Achievements of Royal Arms in Churches in the counties of Somerset, Gloucester and Wiltshire, and the City of Bristol, and he knew the Arms of every family living within thirty miles of Bristol. His eye for detail led him to be the first person to record the anthropophagous figures which appear, often at a great height from the ground, on the external fabric of some churches.

Professor Fawcett leaves a widow, a son, and a daughter, and to these we extend our sincerest sympathy. J. M. Y.